The vertical export of carbon and nitrogen caused by zooplankton diel vertical migration

Thesis

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THE VERTICAL EXPORT OF CARBON AND NITROGEN CAUSED BY ZOOPLANKTON DIEL VERTICAL MIGRATION

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"The Way that can be told of is not an Unvarying Way:
The names that can be named are not unvarying names."
Lao Tzu, "Tao Te Ching" (~300 BC)

Abstract

Fieldwork was conducted at three contrasting sites to test the applicability of an *in situ* technique (ZOOFLUX) for the assessment of the role of zooplankton diel vertical migration (DVM) in the removal of carbon and nitrogen from the surface layer of the ocean to the depths (the active flux). ZOOFLUX relies on the detection of a significant dawn-dusk difference in the carbon and nitrogen weight of migrating individuals (δ). Therefore, its successful application is highly dependent upon the ecology of the migrant species, the level of individual variability in carbon and nitrogen weight (V), and the number of samples that can be collected (n).

At site 1 (the Clyde Sea, western Scotland), *Calanus finmarchicus* and *C. helgolandicus* exhibited a variety of migration patterns, and did not always conform to the 'normal' DVM pattern of up at dusk and down at dawn (NDVM). As a result, δ was variable and V relatively high, while n was relatively low. When δ was non-significant, the probability of making a Type II statistical error (β) was high. In most cases, both the minimum number of samples (n_{min}), and the minimum diel change occurring in carbon and nitrogen weight (δ_{min}), would have needed to be unrealistically high before ZOOFLUX was applicable to these species.

At site 2 (the Sargasso Sea off Bermuda), *Pleuromamma xiphias* and krill (*Thysanopoda aequalis*, *Euphausia hemigibba*, and *E. brevis*) both performed NDVM. At site 3 (Doubtful Sound, New Zealand), *Nyctiphanes australis* performed NDVM at the population level, but some individuals remained at depth during the night, and others at the surface during the day. Despite the more uniform pattern of NDVM at both of these sites, the findings were similar to those at site 1: δ was variable, V relatively high, and n relatively low. Again, this meant that n_{min} and δ_{min} were often unrealistically high.

These findings are discussed in terms of (1) what we can now say about the factors contributing to the active flux, (2) the applicability of the ZOOFLUX technique, and (3) the way forward for future studies. While the ZOOFLUX technique is advocated for future application, it will only prove successful with a prior knowledge of the DVM behaviour of the target species, and the ability to collect interzonal migrants at the critical moments at which they pass both up and down through the pycnocline during the diel cycle.

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Toby Jarvis

To Mum and Dad,
for always being there.

1

ZOOPLANKTON VERTICAL MIGRATION AND GLOBAL BIOGEOCHEMICAL CYCLES: AN INTRODUCTION

1.1 Zooplankton vertical migration

1.1.1 Introduction

We are all aware of migrations in the animal kingdom. From the huge numbers of wildebeest that cross the African plains, to the massive distances covered by Arctic terns and oceanic turtles, animals migrate* in order to satisfy one or more of life's three main driving forces: sex, hunger and survival. Interestingly, however, most of us are unaware of what has to be the largest (in terms of biomass) and most regular animal migration on the planet: the diel (daily) vertical migration of zooplankton, or DVM for short.

The word "zooplankton" is a collective term, used to represent any aquatic animal that is unable to swim horizontally against the prevailing current (from the Greek *planktos*, drifting). Zooplankton typically range in size from ~0.02 to ~20 mm, although some jelly-like organisms, such as pyrosomes (Thaliacea), may form tube-shaped colonies up to an incredible 20 m or so in length. Given that most of the Earth's surface is covered with water (71 %)†, the fact that zooplankton are widespread throughout this environment, and the fact that a substantial proportion of zooplankton species undertake vertical migration (VM) over a range of spatio-temporal scales, it is perhaps not surprising that the scientific literature "bears weary witness" to this phenomenon (Zaret & Suffern, 1976). For example, analysis of *Web of Science* entries for the period 1980-

* **Migrate** vb. to journey between different habitats (from Latin *migrāre* to change one's abode) (Collins English Dictionary).

† This fact has prompted the novelist Arthur C. Clarke to observe, "How inappropriate to call this planet Earth, when clearly it is Ocean". However, although the weight of the oceans is 250 times that of the atmosphere, it is still less than 0.1 % of the weight of the Earth: indeed, if the Earth were 30 cm across, then the average depth of the oceans would be roughly the thickness of a piece of paper. Perhaps the name "Earth" is appropriate after all...

2002 revealed over 1500 publications relating to some aspect of VM in the aquatic environment.

Bayly (1986) described how the French anatomist and palaeontologist Georges Cuvier managed to take enough time off from debating the finer points of evolution with Jean Baptiste Lamarck to make the first recorded observations of VM behaviour (Cuvier, 1817). Almost 200 years have passed since then, throughout which time the scientific literature regarding VM theory has been bombarded with reviews (Russell, 1927; Cushing, 1951; Bainbridge, 1960; Banse, 1964; Hutchinson, 1967; Vinogradov, 1968; Longhurst, 1976; Zaret, 1980; Kerfoot, 1985; Haney, 1988; Lampert, 1989; Pearre, 2003; Hays, in press), notes, comments (both serious and jocular) and reports of endless single- and multi-species studies. Each of these have attempted to describe and/or explain parts of a behavioural "puzzle" (Hardy, 1956) that at first glance seems simple but which, on further examination, shows all the hallmarks of the subtlety, complexity, interaction and variability inherent in any ecological system. The story of VM theory has been, and continues to be, a classic, a real lesson in scientific thinking and process, and a subject to be relished by any ecologist. As Hardy (1956) wrote, "Far from being simple, the more we investigate, the more involved does the plot of the story seem to become".

1.1.2 Some important terminology, and a few questions

"Amplitude" is the word given to describe the distance travelled during migration. Those animals that spend the majority of their time in the surface layer are known as "surface-dwellers" or "epizooplankton", while those that spend part of their time in the surface layer and another part in the deeper layers are known as "bathypelagic" or "interzonal" species (see Vinogradov, 1968, and references therein). The terms

"epizooplankton" and "interzonal" will be used throughout this thesis.

Even a brief consideration of zooplankton VM behaviour will raise a barrage of questions. Where and when do migrations take place? Which organisms migrate, and how many are involved? How fast do they migrate? Why do they migrate? Are there any ecological or environmental impacts as a result of vertical migration? All of these questions have been addressed in one way or another, in particular that of why zooplankton migrate, and VM theory now has an impressive grounding in the literature. A number of investigations over the last decade or so have begun to address the last question raised, that of the potential ecological and environmental impacts of VM behaviour, which perhaps reflects current trends in global-scale environmental awareness and thought.

1.1.3 Where and when: spatio-temporal patterns of vertical migration

In terms of where migrations occur within the water column, the amplitude of VM varies from tens of centimetres to hundreds of metres, and VM may well take place at all depths down to the abyssopelagic zone (see Figure 84, the "migration ladder", in Vinogradov, 1968). However, Longhurst (1976) questioned some of the data upon which this ladder was based, and concluded that 1700 m "appears to be the greatest depth for which we have good evidence of diel migration". In terms of where migrations occur over the world's oceans and lakes, Vinogradov (1968) suggested that such behaviour is ubiquitous, but especially prominent in highly productive tropical areas.

As for when migrations occur, VM has been observed over diel, seasonal and ontogenetic (developmental stage) time scales (DVM, SVM and OVM, respectively). DVM typically involves ascent into the food-rich surface layer and descent into the

relatively food-depleted depths over the course of the diel cycle. When descent and ascent correspond closely to dawn and dusk, respectively, this pattern is known as 'normal' DVM (NDVM). NDVM is by far the most commonly observed pattern in migrating zooplankton, both in marine and freshwater habitats, and is backed up by countless observations in the literature. Cushing (1951) suggested a "general picture" of DVM by splitting the process into five parts: (1) ascent from the day depth, (2) departure from the surface at or around midnight (midnight sinking), (3) return to the surface just before dawn (dawn rise), (4) sharp descent around first light (dawn descent), (5) variable day depth. In a number of cases, a 'reverse' pattern has been observed (RDVM), whereby individuals are actually found shallower during the day and deeper at night (e.g. Ohman *et al.*, 1983; Bayly, 1986; Neill, 1990; Herwig & Schindler, 1996). While Bayly (1986) described this pattern as an "enigma", he also pointed out that it is often the study of such exceptions that allows us to prove the rule.

Seasonal vertical migration (SVM) is said to have occurred when a given species is to be found at different depths at different times of the year. Ontogenetic vertical migration (OVM) is said to have occurred when a given species is to be found at different depths at different stages in its life history. The boundaries between SVM and OVM are often blurred, as Longhurst (1976) suggested: "Since most, though not all, plankton species which show these sorts of migrations have several generations each year it is not useful to separate ontogenetic from seasonal migrations, since so frequently they are one and the same thing". Banse (1964) had made similar suggestions previously. While somewhat of an academic point, an example of this is evident from studies of the copepod *Calanus finmarchicus* in the northeast Atlantic (e.g. Marshall & Orr, 1955; Hirche, 1983; Hind *et al.*, 2000). The population remains at depth as an overwintering strategy, resuming DVM the following spring with the onset of

favourable conditions, and this whole cycle may be regarded as SVM. The fact that the population typically overwinters as the sub-adult stage CV, and moults into the adult stage CVI in the spring, means that this pattern can also be regarded as OVM. In general, however, younger stages of a species tend to remain closer to the surface than older stages (OVM), a pattern that is well supported in the literature (e.g. Brinton, 1962; Vinogradov, 1968; Huntley & Brooks, 1982; Neill, 1992; Hays, 1995).

Examples of SVM include those of Mackintosh (1937) for a number of Antarctic zooplankton species, Williams & Conway (1984) for *Calanus helgolandicus* in the Celtic Sea, and Bradford-Grieve *et al.* (2001) for *Neocalanus tonsus* off New Zealand. A particularly useful dataset for the study of spatio-temporal trends in VM behaviour is that derived from the Continuous Plankton Recorder (CPR) survey (Hardy, 1939), which Hays *et al.* (1995) and Hays (1995, 1996) made use of to assess the VM patterns of *Metridia* and other species in the North Atlantic and North Sea. Spatially, the greatest variability in VM was seen with latitude, particularly with regard to seasonal patterns, although the general rule that younger stages are found shallower seemed to hold in all areas. In the polar summer, DVM may be completely stopped (e.g. Digby, 1961a), but this is not always the case (e.g. Dale & Kaartvedt, 2000; Fortier *et al.*, 2001). In general, however, one sees more evidence of seasonality at higher latitudes. At lower latitudes, only certain areas show marked seasonality, such as the Californian and West African coastal upwellings (Longhurst, 1976).

1.1.4 How many and who: the global significance of vertical migration

There are many who would agree that zooplankton VM is the largest animal migration on the planet. For example, Enright (1978) wrote: "In my opinion, the most important kind of animal migration...is the diurnal vertical migration of zooplankton. Whether

viewed in terms of the number of species involved, the number of animal-kilometres travelled per year, or simply the gross biomass that is transported, these vertical migrations are probably the most impressive of the temporally coordinated mass movements of animals known to science". When faced with the statistics, it is not hard to justify this. Of the 510 million km^2 of the Earth's surface, 150 million km^2 (29 %) are accounted for by land, 350 million km^2 (69 %) by seawater and 10 million km^2 (2 %) by freshwater. In the top 500 m of the oceans, the global biomass (wet weight) of zooplankton and micronekton (primarily small fish) combined has been estimated at 4 Pg*, which equates to ~0.17 Pg C (Bogorov *et al.*, 1968; Moiseev, 1971). Longhurst *et al.* (1990) estimated that 10 to 20 % of this biomass will perform DVM, meaning that, globally, up to nearly a billion tons (1 Pg) of zooplankton, representing 35 million tons (0.035 Pg) of carbon, may be entering and leaving the surface waters every single day. This is a significant and regular shift of biomass indeed.

For each of the numerous and varied phyla represented by the zooplankton, there is at least one species which has been observed to undertake VM (Huntley, 1985). As Longhurst (1976) wrote, "predators as different as siphonophores and chaetognaths, herbivores as different as copepods and euphausiids, and animals ranging in size from tintinnids to large oceanic squid are all now known to migrate vertically". Of these phyla, it is fair to say that the crustacea are the dominant vertical migrants (e.g. Bainbridge, 1960, cited observations from 156 species). Of the crustacea, the dominant migrant groups are copepods (in particular the Calanoida) and krill (Euphausiacea).

* 1 Petagram (Pg) = 1×10^{15} grams (1000 billion, or 1 quadrillion grams). This is a commonly used unit when describing global-scale amounts of material. Another commonly used, and indeed synonymous, unit is the Gigaton (Gt), where 1 Gt = 1×10^{9} tons (1 billion tons) = 1 Pg.

1.1.5 How fast: understanding fitness costs, motivation, and timing

Enright (1977a) suggested "there are various reasons for an interest in the speed at which small aquatic organisms propel themselves through the water". These include evaluating the energy costs associated with VM behaviour (e.g. Vlymen, 1970), understanding the motivations of individuals and/or populations, and knowing how long it takes for an individual or population to complete its migration pattern. The classic text on this subject is that of Hardy & Bainbridge (1954), whose ingenious "plankton wheel" allowed swimming speeds to be measured in the laboratory. This study revealed speeds ranging from 3.24 m h^{-1} (0.9 mm s^{-1}) in the small copepod *Paracalanus parvus*, to 215.1 m h^{-1} (60.2 mm s^{-1}) in the krill *Meganyctiphanes norvegica*. Downward speeds are often seen to be faster than upward speeds, no doubt due to the additional velocity attributable to gravity (e.g. see Table 64 in Mauchline, 1998, for data and references on passive sinking in copepods). *In situ* migration speeds are typically inferred from net-derived changes in the vertical distribution of a population over time (e.g. Enright, 1977a; Wiebe *et al.*, 1992), providing velocity estimates of comparable magnitude to those from the plankton wheel. However, it is well understood that such estimates may not represent the swimming speeds of individuals (Pearre, 1979a), an issue that is being addressed through the use of alternative instruments such as acoustic profilers (e.g. Pleuddemann & Pinkel, 1989; Tarling *et al.*, 1998, 2002).

1.1.6 Why: immediate causes and biological advantages

So why do zooplankton undertake such movements? Put another way, what are the causes of VM behaviour? As Vinogradov (1968) explained, "In discussing the causes of vertical migrations it is necessary to distinguish between the two aspects of the question: (1) the immediate causes of the migration which directly stimulate the rise and

descent of the zooplankton; (2) biological advantages which the vertical migration brings to planktonic animals". Immediate causes are known as 'proximate', or 'causal' factors, while biological advantages are known as 'ultimate', or 'functional' factors.

Proximate factors

Proximate factors may be divided into two categories, depending on whether the stimulus is endogenous (internal) or exogenous (external). Endogenous cues arise from an internal 'body clock' (e.g. Barkai & Leibler, 2000) that, in turn, may or may not be interacting with a hunger response. Exogenous cues arise from the physical and chemical environment surrounding an aquatic organism, and include light, temperature, salinity, dissolved oxygen, hydrostatic pressure, and food availability. Of these, only light, dissolved oxygen (as a product of photosynthesis) and food availability change in such a way as to be realistically considered as proximate stimuli for the movement of individuals.

The evidence for endogenous rhythms in aquatic animals has been well demonstrated, and the subject comprehensively reviewed by Harris (1963). In terms of the body clock acting as a proximate factor in VM, however, the results are equivocal (Enright & Hamner, 1967). Vinogradov (1968) cited evidence from studies both for and against the importance of an internal clock, and concluded that, in fact, light is a much stronger factor. Certainly this has been supported by observations of sudden upward migration when light levels fall, as during a solar eclipse (e.g. Petipa, 1955; Backus *et al.*, 1965) or during experimental manipulations on *Daphnia* (Loose, 1993). Huntley & Brooks (1982) suggested that the internal clock in *Calanus pacificus* might act as a secondary cue, with hunger acting as the primary cue that is able to override the internal clock as part of a cost/benefit analysis. Simard *et al.* (1985) essentially agreed with this,

with their results of feeding studies on *Calanus finmarchicus* supporting the suggestion of an *in situ* grazing rhythm related to hunger and satiation. In a more 'traditional' way, Buchholz *et al.* (1995) spoke of light acting as a body-clock *zeitgeber* ('time-giver', or synchroniser) for the VM behaviour of *Meganyctiphanes norvegica*. An opportunistic study during a lunar eclipse (Tarling *et al.*, 1999a) helped to confirm this: the sudden unpredictable darkening of the moon acted to confuse the behavioural pattern of midnight sinking, which otherwise seemed to be controlled by an endogenous rhythm in synch with the lunar cycle. Rudjakov (1970) suggested that VM is not the result of adaptation to a planktonic mode of life, but rather the result of circadian rhythms controlling sinking and swimming activity. However, in the context of other factors such as predator avoidance (see below), for the internal clock to be a primary cue might mean that an individual adopts the maxim 'better dead than hungry' in certain cases, a trade-off which is unlikely to say the least. It would seem that, if the effect of an internal clock is indeed present, its importance varies between species (Forward, 1988), and many would suggest that it is fairly weak in its control over migratory behaviour (e.g. Harris, 1963; Enright & Hamner, 1967; Enright, 1970; Ringelberg, 1995a; Mauchline, 1998).

As for exogenous factors, a correlation between light and zooplankton behaviour has been noted and investigated in countless studies (e.g. Rose, 1925; Russell, 1926; Clarke, 1933; Kampa & Boden, 1954; Clarke & Backus, 1956; Buskey *et al.*, 1989; Frank & Widder, 1997; Gal *et al.*, 1999; Strömberg *et al.*, 2002). In particular, it is the intensity of downwelling irradiance that quite evidently links to facets of migration such as timing (dusk ascent, dawn descent) and amplitude (e.g. Stearns & Forward, 1984; Buskey *et al.*, 1989; Jerling & Wooldridge, 1992).

One might also ask what other features of the downwelling irradiance the

zooplankton might be responding to. A number of investigators have suggested that individuals follow particular light intensities, or isolumes, as they move up and down in the water column over the daily cycle (e.g. Michael, 1911; Bogorov, 1946; Digby, 1961a; Boden & Kampa, 1967). However, the so called 'light preferendum model', or 'isolume hypothesis', has not been reproducible, and Roe (1983, 1984) expressed doubt that most species of zooplankton are capable of attaining the swimming speeds necessary to follow a narrow isolume during sunrise or sunset. That said, Mauchline (1980) and Widder & Frank (2001) suggested that certain faster-swimming species such as krill and sergestid shrimps could be controlled in this way. Another explanation is that vertical migrants are not following absolute light levels, but are responding to particular rates of change of light intensity (e.g. Bary, 1964; Ringelberg, 1964, 1991; Andersen & Nival, 1991). This 'rate of change hypothesis' has been reviewed and supported by Ringelberg (1995b, 1999). He showed how relative changes in light intensity are greatest just before dawn and just after sunset, and that it is these peaks that zooplankton are using as cues to begin VM. This hypothesis is certainly more reproducible than the isolume hypothesis, and has received the widest support among the scientific community.

Considering temperature as a proximate cue, Vinogradov (1968) thought that diel changes in the mixed layer would be too subtle to be of any consequence, but cited evidence for its interaction as a modifying factor (e.g. Rose, 1925; Vinogradov & Voronina, 1964). Most noticeable to migrating zooplankton is the large differential at the thermocline, where temperature may change by as much as 15 °C over a relatively short depth. Many zooplankton species are known to cross the thermocline, but variability in response to this feature is seen between species (e.g. inter-genus differences in ostracods: Angel, 1968) and within species over their ontogeny. Also,

more often in high latitudes, it may even act as a complete barrier (e.g. Buchholz *et al.*, 1995; Bergström & Strömberg, 1997). Mauchline (1998) suggested that migratory behaviour is only modified by temperature when it is at or near the distributional defining limits for a species. An example of this has been seen in the distribution of two similar species, *Calanus finmarchicus* and *C. helgolandicus*, in the Celtic Sea (Williams, 1985). *C. finmarchicus* is at the southern end of its range here, and may be confined to the cooler waters below the thermocline, while *C. helgolandicus* is near the northern end of its range and may be confined to the warmer surface waters.

As with temperature, one might expect salinity to present a problem only when near its upper distributional limit. Below this level, one may expect salinity only to have a modifying influence on behaviour. Salinity preferences vary greatly from species to species, largely dependent on their morphological and physiological adaptations, such that one might expect to see interspecific variability in response to salinity gradients. However, the literature in this area is surprisingly thin, and Longhurst (1976) cited Hansen & Dunbar (1970) as giving "one of the few satisfactory accounts of a constraint due to a salinity discontinuity". Buchholz *et al.* (1995), on the other hand, found salinity to exert no effect at all on the VM behaviour of *Meganyctiphanes norvegica*. A lack of many more examples suggests that this might be a factor requiring further study.

The presence of an oxygen minimum layer in certain areas represents a potential barrier to VM. Often this layer is accompanied by the production of hydrogen sulphide (H_2S), as, for example, in the Black Sea (Caspers, 1957). Both Ivanenkov & Rozanov (1961) and Longhurst (1967) suggested that plankton may be excluded from areas where oxygen is below 0.2 ml l^{-1}, or where there is H_2S present, and Longhurst (1967) showed that between 0.2 and 0.5 ml l^{-1} one might find diel and seasonal migrators during their deeper-layer residence. Investigations of *Meganyctiphanes norvegica* in

Gullmarsfjord, Sweden (Spicer *et al.*, 1999; Strömberg & Spicer, 2000) have shown that this species is only able to survive hypoxia during deep daytime residence due to the reduced temperature at depth and the ability to perform anaerobic respiration.

Vinogradov (1968) gave one of the few reviews of hydrostatic pressure as a proximate cue for VM. Many studies have found no response at all by marine plankton to pressure (e.g. Hardy & Paton, 1947; Hardy & Bainbridge, 1954; Knight Jones & Qasim, 1955), while the statistical considerations of Moore (1955), Moore & Corwin (1956) and Moore & Bauer (1960) pointed to VM behaviour as being a response to the combined effect of light, temperature and pressure, factors that all relate to each other in the sea. This is a compelling suggestion, since it makes sense intuitively that any response elicited by an organism be the result of a combination of the factors making up its environment. One further point raised by Vinogradov (1968) was how animals lacking a gas cavity might be able to detect pressure changes. Digby (1961b) hypothesised a thin layer of gas surrounding the organism, while Enright (1963) showed that, in fact, pressure might be felt since the compressibility of a zooplankter may differ by 15-40 % from that of seawater.

In terms of food availability as a proximate cue, Longhurst (1976) cited evidence that zooplankton often aggregate in areas of high food availability (e.g. Anderson *et al.*, 1972; Mullin & Brooks, 1973). Vinogradov (1968) spoke of VM behaviour in terms of rising to eat only when other adverse factors are at a threshold minimum, while Mauchline (1998) highlighted studies that showed interspecific variability in the strength of food availability as a proximate cue (e.g. Yen, 1985; Harris, 1988). The "hunger/satiation hypothesis" (e.g. Simard *et al.*, 1985; Gibbons, 1993), or HSH for short (Pearre, 2003), suggests that the trophic state of an organism can sometimes result in VM movements being initiated in the absence of light and other cues. In this

scenario, it is evident that the endogenous cues of hunger and satiation are inextricably linked with the exogenous cue of food availability: hunger and satiation will help an individual to decide when to move, while the location of available food will help it decide where to move to. In a similar way, the body condition of an organism can also influence its patterns of behaviour as a function of both endogenous and exogenous cues. A number of studies have demonstrated the influence of body condition on zooplankton VM (e.g. Bollens & Frost, 1991; Tarling *et al.*, 1999b; Hays *et al.*, 2001a), but Hays *et al.* (2001a) expressed surprise "that the impact of body condition on DVM behaviour has not been more extensively explored".

Ultimate factors

It is widely realised that we cannot hope to find a single, all-encompassing factor to explain how zooplankton benefit by performing VM. Nor should we expect to, given the diversity of species and habitats in which VM occurs. Most of the hypotheses generated on this subject have considered DVM, and in particular the behaviour of mesozooplankton (size range 0.2 to 20 mm: see e.g. Sieburth *et al.*, 1978) that are known to feed either herbivorously or omnivorously predominantly in the surface layer (Hays, in press). The various hypotheses that have been put forward were categorised by Fiksen (1997) into (1) group selection hypotheses, (2) metabolic advantage hypotheses, (3) the predator avoidance hypothesis, and (4) other hypotheses.

Group selection hypotheses propose that VM allows the most efficient use of phytoplankton production to be sustained (Hardy, 1956; Kerfoot, 1970; Lane, 1975; Tande, 1988; Unstad & Tande, 1991). However, as Fiksen (1997) also pointed out, these are based on group selection arguments that are not supported by Darwinian logic: they assume that individuals behave in a way that maximises benefit to the group, but

not necessarily to themselves.

Metabolic advantage hypotheses propose that VM confers an energetic bonus to an individual. In the case of DVM, McLaren (1963) suggested that feeding in the warm food-rich surface layer at night and resting in the cooler deep waters by day would reduce energy loss. On the subsequent understanding that development is also slowed at colder temperatures, McLaren (1974) modified his idea to include a possible demographic (population) advantage: females may grow larger at colder temperatures and so produce more offspring. However, just the opposite has been observed, both in the field (Stich & Lampert, 1981) and in the laboratory (Swift, 1976; Orcutt & Porter, 1983; Stich & Lampert, 1984). While the "starvation avoidance hypothesis" proposed by Geller (1986) might support the metabolic/demographic concept in certain circumstances, Lampert (1989) expressed his doubts as to the general validity of these hypotheses. A new model suggested by Enright (1977b) and tested by Enright & Honegger (1977), specifically that nocturnal feeding would increase zooplankton growth by enhancing phytoplankton production, generated an interesting and amusing debate (Enright, 1979; Koslow, 1979; Miller, 1979; Pearre, 1979b). However, Lampert (1989) again expressed doubts, and it is now generally thought that vertical migration is actually energetically disadvantageous (but see Williamson *et al.*, 1996).

The predator avoidance hypothesis has been developed primarily to explain DVM, and proposes that this ubiquitous pattern of behaviour allows zooplankton to avoid visual predation in the surface layers by taking temporary refuge in the darker depths during the day. Framed in terms of cost versus benefit, the cost of DVM is a period of reduced feeding while at depth, while the benefit is a reduced probability of predation. In this scenario, migrants therefore adhere to the maxim "better hungry than dead" (Kremer & Kremer, 1988). This simple hypothesis has developed from field and

laboratory observations (Zaret & Suffern, 1976; Stich & Lampert, 1981; Williamson & Magnien, 1982; Ohman *et al.*, 1983; Vourinen *et al.*, 1983; Gliwicz, 1986; Frost, 1988), and has received strong support from numerous models (e.g. Iwasa, 1982; Clark & Levy, 1988; Ohman, 1990; Rosland & Giske, 1994). *A priori* predictions of the predator avoidance hypothesis are (1) that individuals will ascend at dusk and descend at dawn, (2) that DVM will be more pronounced in more conspicuous individuals, and (3) that the amplitude of migrations will vary with the abundance and activity of planktivorous fish (Lampert, 1989). These predictions have each been fulfilled in numerous studies, often by means of particularly elegant experiments (see references in Hays, in press), and the predator avoidance hypothesis has gained strong acceptance within the scientific community. Interestingly, even the apparently anomalous pattern of RDVM can be explained in terms of predator avoidance, with this behaviour thought to be the result of avoidance of invertebrate predators which themselves are performing NDVM (e.g. Ohman *et al.*, 1983).

Other hypotheses include the avoidance of damaging ultraviolet radiation (e.g. Leech & Williamson, 2001), and the potential for horizontal displacement or retention (e.g. Hardy, 1936; Hill, 1998). Neither of these, however, have the same widespread applicability and intuitive appeal as the predator avoidance hypothesis. Hays (in press) summed up what we can say to date about the ultimate causes for DVM: "In short, while there are certainly a number of reasons for why mesozooplankton undertake DVM, probably the ultimate reason in most cases is predator evasion".

1.2 Global biogeochemical cycles

1.2.1 What is biogeochemistry?

Our planet can be thought of as a series of compartments into which the 105 known elements are variously distributed. If one ignores minor fluxes to and from space, then the sum of these compartments can be seen to represent a closed global system. The main non-living (abiotic) compartments are the atmosphere, the lithosphere and the hydrosphere. Elements within these are said to exist in inorganic form (e.g. carbon in carbon dioxide gas, calcium in limestone rocks, nitrogen in dissolved nitrate). A final compartment, the biosphere, represents all those elements that comprise living, dead and decaying material. Elements within the biosphere are said to exist in organic form (e.g. carbon in cellulose or lipids, nitrogen in protein, phosphorus in adenosine triphosphate). Both organic and inorganic elements undergo constant change and redistribution via a series of biological, geological and chemical processes. The study of these processes, and in particular the fluxes (flows) of elements between compartments, is aptly known as "biogeochemistry"*.

1.2.2 Why study biogeochemistry?

Many geological and chemical processes would still occur in the absence of life. For example, volcanoes would still release sulphur from the lithosphere into the atmosphere, and lithospheric calcium would still be eroded into the hydrosphere via weathering. However, the various activities of the biosphere (and that includes humans) do have a profound influence on the patterns of flux within the global system, and are therefore of major significance within considerations of biogeochemical processes.

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* **Biogeochemistry** n. the science that deals with the relation of earth chemicals to plant and animal life (Webster's Third New International Dictionary).

Recognising that every biogeochemical process is cyclical over some time-scale, it might well be asked why it is necessary for us to study them: if these processes are cyclical, then surely the global system exists in a dynamic equilibrium and functions essentially as a cybernetic*, self-regulating entity? Well, the unfortunate position that humankind finds itself in, with our ability to recognise that our actions have consequences, is that we are unsure whether our everyday activities are systematically affecting the biosphere in an adverse way, or whether we are simply an integral part of its 'natural' cycles. Are the various anthropogenic (man made) changes that we see in our environment merely part of the natural scheme of things, or are we actually damaging our environment irreversibly? Lovelock (1979) took an interesting view. In his "Gaia Theory", in which Gaia represents the planet Earth (named after the Greek goddess of the Earth), he wrote, "it may be that...our technology will in the end prove destructive and painful for our own species, but the evidence for accepting that industrial activities...may endanger the life of Gaia as a whole, is very weak indeed". The issue, however, is less whether our activities will completely wipe out life, and more whether they will degrade its quality as we know it. Viewed in these terms, the study of biogeochemical cycles is justified: if we can understand and monitor the way in which the most influential elements are being distributed within and between compartments, then we are more likely to be able to detect adverse anthropogenic changes and, as self-elected custodians of the planet, discover ways of mitigating them.

* **Cybernetics** n. the branch of science concerned with...the extent to which useful comparisons can be made between man-made and biological systems (from Greek *kubernētēs* steersman, from *kubernan* to steer, control). See also **feedback** (Collins English Dictionary).

1.2.3 Anthropogenic impacts on biogeochemical cycles

Two of the most important elements to study within the context of biogeochemical cycles are carbon (C) and nitrogen (N). Carbon is of particular interest to biogeochemists because, as carbon dioxide (CO_2) in the atmosphere, it acts to trap long-wavelength (infrared) radiation reflected from the Earth's surface. Moreover, it is general consensus that the effect of this trapping, the so-called 'greenhouse effect', while dependent on a number of complex feedbacks, is probably an overall warming of the Earth's atmosphere. As a consequence of this potentially serious effect, a major proportion of biogeochemical studies have focused, either directly or otherwise, on the cycling of carbon. In short, these studies have shown that concentrations of atmospheric CO_2 have increased by more than 30 % since the 'industrial revolution' in the mid-18th century (Barnola, 1999; Keeling & Whorf, 2000), with these increases being responsible for more than half of the 0.6±0.2 °C global warming observed during the past century (Houghton, 2001). Furthermore, we can be sure that the bulk of these increases are due to human activities: for example, anthropogenic inputs of carbon (as CO_2) to the atmosphere averaged ~6 Pg C y^{-1} during the 1980s (Schimel *et al.*, 1995). "Humankind thus appears to be playing a significant role in altering Earth's climate" (Sarmiento & Gruber, 2002). Other important anthropogenic greenhouse gases contributing to this temperature rise include methane (CH_4), nitrogen oxides (NO, N_2O) and chlorofluorocarbons (CFCs).

So how has the evidence for these carbon-based anthropogenic impacts been gathered, and what is being done with the data? Firstly, measurements of atmospheric CO_2 concentrations dating back an incredible 420,000 years have been made from bubbles of air trapped in Antarctic ice cores (Petit *et al.*, 1999). In addition, measurements have been made on air samples collected at regular (~monthly) intervals

from Hawaii since 1958 (Keeling & Whorf, 2000). These studies have shown that CO_2 concentrations were around 280 parts per million (ppm) for several thousand years, before increasing steadily around the early 1800s to the ~370 ppm that we see today. Indeed, measurements from ancient ocean sediments would suggest that today's levels haven't been seen even within the last 20 million years (Houghton, 2001). Discrepancies between observed increases in atmospheric concentrations, and those that one would expect to see based on the known emissions from fossil-fuel burning and changes in land use, have pointed to the existence of significant 'sinks' for anthropogenic carbon. These sinks have been identified as the terrestrial biosphere (plants and soils) and the ocean, and much work has been carried out to quantify their relative importance and functional mechanisms. Ongoing research continues to improve our understanding of the many terrestrial and oceanic processes, which, as Sarmiento & Gruber (2002) explained, "is key to predicting, and hopefully mitigating, the future impact of anthropogenic CO_2". The data thus generated are being used in a variety of models to predict potential atmospheric CO_2 concentrations under a variety of anthropogenic emissions scenarios (see e.g. Cox *et al.*, 2000, and references in Sarmiento & Gruber, 2002). The present situation has been summarised by Sarmiento & Gruber (2002), who wrote, "although we don't yet fully understand the global carbon cycle, it's safe to say there are no magic bullets in the carbon sinks to rescue the world from high atmospheric CO_2 levels any time in the next few centuries".

As for nitrogen, Libes (1992) explained, "though the...nitrogen cycle may have been in a steady state, human activities are likely to have caused a significant enough change in fluxes so as to perturb any natural balance". Nitrogen is important as a controlling nutrient in biological productivity. Given the importance of the biosphere within the global system, nitrogen is therefore particularly influential, for example, in processes

that regulate climate (e.g. Falkowski, 1997) and the formation of fossil-fuel deposits. As it exists in a variety of oxidation states, the biogeochemical cycling of nitrogen can be particularly complex. The effect of human activities appears to have been to increase some of the global nitrogen fluxes. For example, dinitrogen (N_2), the most common gas in the atmosphere, is being 'fixed' (i.e. combined with other elements) by humans at a rate that is roughly equal to that of the rest of the terrestrial biosphere. The bulk of this fixation occurs in the industrial production of ammonia via the "Born-Haber" process. While the fertilisers so produced have increased the biomass of the terrestrial biosphere, sewage outputs and poor agricultural practices have resulted in massive inputs of fixed nitrogen to the hydrosphere. These inputs may increase primary production to unnaturally high levels, a process known as eutrophication, thereby increasing the biological oxygen demand and potentially altering ecosystems. An upshot of this, however, is that the increased productivity may help to consume more CO_2 and thereby reduce atmospheric levels of this greenhouse gas. Finally, the combustion of fossil fuels, and the burning of terrestrial biomass, act to release NO and N_2O (nitrogen oxides, sometimes referred to as 'NOx') into the atmosphere. Here they act as greenhouse gases, as well as contributing to acid rain, the formation of ozone in the lower atmosphere and its destruction in the upper atmosphere.

An encouraging fact, however, is this: we no longer live in a world in which economic growth and the use of non-sustainable fossil fuels are mutually exclusive. Indeed, the technology already exists for an 'energy revolution'. Hopefully it won't be long before a general acceptance is gained for the idea that we can profit just as much, if not more, from the widespread use of more sustainable energy sources.

1.2.4 The role of the oceans in biogeochemical cycles

The oceans are currently estimated to contain ~38,000 Pg C (see Figure 1 in Sarmiento & Gruber, 2002). This represents ~85 % of global carbon, the majority of which is present as bicarbonate ions ($2HCO_3^-$: this is classified as dissolved inorganic carbon, DIC) below the mixed layer. The oceans are also estimated to contain ~23,000 Pg N (see Table 24.2 in Libes, 1992), the majority of which is present as dissolved N_2. In contrast to carbon, however, this represents only ~0.01 % of global nitrogen. Most of the Earth's nitrogen is actually contained in the lithosphere, primarily within rocks.

Two processes contribute to the ultimate storage of bicarbonate in the ocean's interior: the solubility pump, and the biological pump. In the solubility pump, atmospheric CO_2 dissolves into the surface layer of the ocean, thereby contributing to the pool of DIC. This occurs most rapidly in the colder surface waters nearer to the poles. Since cold water is also dense, these DIC-rich waters sink to the depths, where they circulate around the deep oceans for up to 1000 years or more before returning to the surface. The most significant regions contributing to this 'thermohaline circulation', or 'conveyor belt', are the waters around Antarctica, and the far northern Atlantic Ocean. The continual removal of DIC-rich surface waters to depth maintains the air-sea gradient in CO_2, and allows the air-sea flux to continue. In the biological pump (Longhurst & Harrison, 1989; Longhurst, 1991), DIC in the sunlit surface waters is converted (fixed) into organic compounds via the photosynthetic activity of the phytoplankton. This process, known as "primary production", is controlled by the availability of light and nutrients, and the activity of predators (see e.g. Flynn, 1988, 1989). Global estimates for the fixation of DIC via marine primary production are around 35-50 Pg C y^{-1} (see Table 1 in Holligan, 1995). The limiting nutrient to phytoplankton growth is typically dissolved inorganic nitrogen (DIN) in the form of

nitrate (NO_3^-), but this is not always the case (see e.g. Boyd & Law, 2001, for an introduction to studies of iron as a limiting 'nutrient' in the Southern Ocean). The organic carbon compounds produced by photosynthesis may be passed on, altered and recycled in the surface mixed layer by the biota (typically, phytoplankton → zooplankton → higher predators), or find their way into the depths where they may be altered and sequestered (stored), for potentially long periods of time. The removal of material from the surface layer to the depths, whether organic or inorganic, is referred to as 'export flux'. It is evident that, as long as there is a net export flux of carbon, the ocean will continue to 'breathe in' atmospheric CO_2, and therefore act as a sink for anthropogenic inputs of this greenhouse gas (Sarmiento & Siegenthaler, 1992).

The oceanic nitrogen cycle is altogether more complex, and linked strongly to the activities of the marine biota. Sources of fixed nitrogen to the oceans (i.e. those forms of nitrogen that are directly usable by the biota) include rivers (fluvial input), rainfall (precipitation), diffusion from the sediments, hydrothermal vents, and wind-blown dust (aeolian input, or dry deposition). Only a handful of marine organisms are capable of directly fixing atmospheric N_2 that has dissolved into the surface waters. Nitrogen may leave the oceans as gaseous N_2 generated via denitrification (bacterial respiration), or via gaseous N_2O fluxes from the sea surface. The removal and consumption of marine biota by fishing represents another potentially significant loss term. Within the water column, fixed nitrogen is incorporated into the food chain via photosynthesis. Primary production is sustained in the long term by the upward turbulent flux of DIN (as dissolved NO_3^-) from below the mixed layer. This is known as "new" production, in contrast to "regenerated" production which is driven by NO_3^- and ammonium (NH_4^+) recycled by the biota within the mixed layer (Dugdale & Goering, 1967). As with carbon, organic nitrogen compounds produced by photosynthesis may continue to cycle

within the mixed layer, or they may be exported to depth.

Oceanic export fluxes

Export fluxes of biogeochemically influential elements such as carbon and nitrogen have important implications in the mitigation of adverse anthropogenic activities. The inception of a number of large-scale past and present oceanographic sampling programmes, for example the Biogeochemical Ocean Flux Study (BOFS Scientific Steering Group, 1989) and the Joint Global Ocean Flux Study (JGOFS), is testament to the recognition of this fact by the international scientific community. Evidence from the field has shown that export flux may occur in one of three ways: (1) passive particulate material flux, (2) passive dissolved material flux, and (3) active particulate and dissolved material flux (Figure 1.1).

The passive flux of particulate material

Conventional wisdom maintains that the rapid sinking of large particles is the dominant export flux (e.g. Agassiz, 1888; McCave, 1975; Suess, 1980). Furthermore, this so-called 'passive' flux, essentially a 'rain' of particulate material, or 'marine snow' (including faecal pellets, moults and corpses, larvacean houses and general detritus), has been suggested to balance new production over sufficient time and space scales (Eppley & Peterson, 1979). As a consequence of its assumed importance, the passive flux has been extensively studied throughout the world's oceans via the deployment of particle interceptor (sediment) traps at various depths in the water column (e.g. Wiebe *et al.*, 1976a; Knauer *et al.*, 1979; Rowe & Gardner, 1979; Shanks & Trent, 1980; Urrere & Knauer, 1981). Global estimates for the passive flux of particulate organic carbon (POC) from the upper ocean are around 3.5 Pg C y^{-1} (Sundquist, 1985).

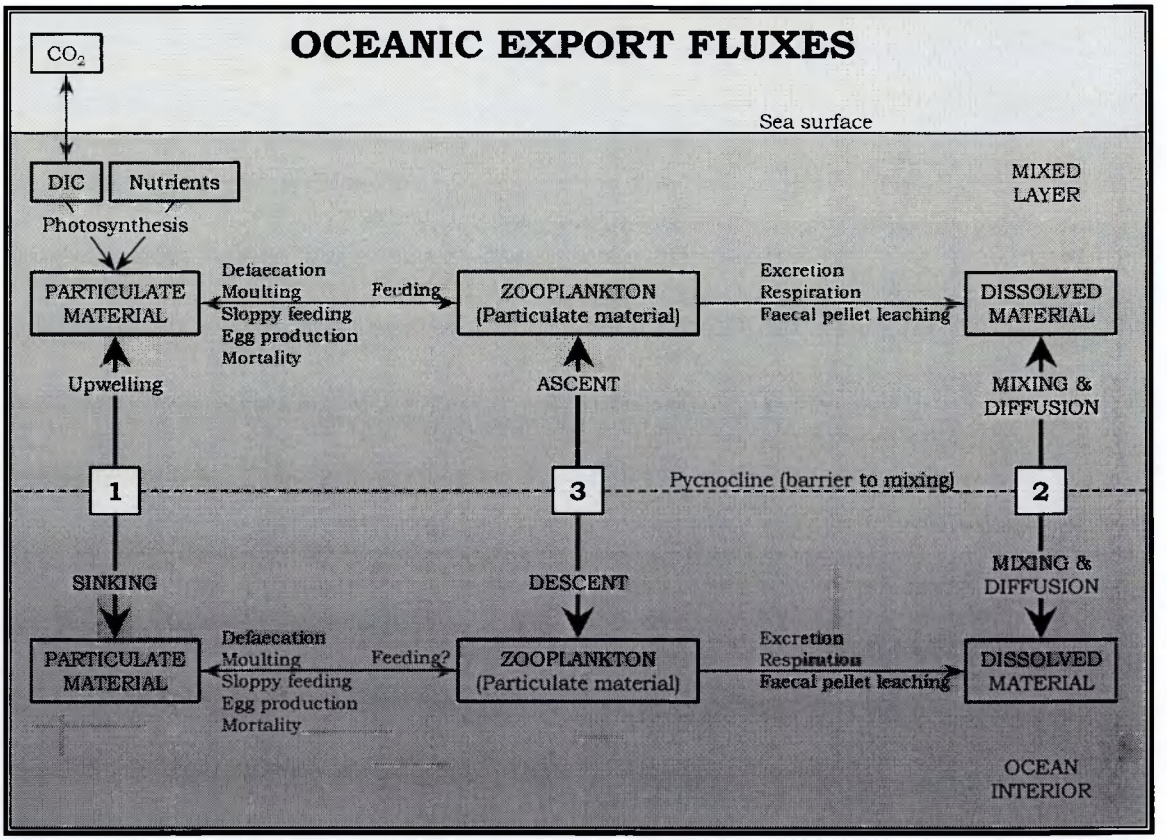

Figure 1.1 A simplified diagrammatic representation of the three main routes by which particulate and dissolved material may be exported from the surface layer of the ocean to depth (see text for details). DIC = dissolved inorganic carbon.

The passive flux of dissolved material

More recently, there has been increasing interest in other potential export fluxes, fuelled in part by an inability to close carbon and other budgets using passive flux assessments alone (e.g. Michaels *et al.*, 1994). In addition to the passive particle flux, it has been suggested that dissolved material may be exported via diffusion and mixing processes (e.g. Toggweiler, 1989; Copin-Montégut & Avril, 1993). This has since been confirmed by, for example, Carlson *et al.* (1994) and Ducklow *et al.* (1995), who demonstrated the importance of the annual passive flux of dissolved organic carbon (DOC) in the subtropical north Atlantic. Following improvements in the precision and accuracy of oceanic DOC measurements (e.g. Benner & Strom, 1993), Carlson *et al.* (1994) showed

how the export of DOC from the surface layer via winter mixing off Bermuda is equal to, or even greater than, the measured passive particle flux. In addition, they proposed that their observations at this site should be applicable to "other temperate, sub-polar and continental-shelf regions of the world ocean which exhibit convective mixing and vernal restratification".

The active flux of particulate and dissolved material

A third route for the export of material from the surface layer of the ocean is via the VM of interzonal species. In short, the loss of waste products and expended resources while at depth may result in the net export of surface-derived material into the ocean's interior. It is evident that this has the potential to occur on varying time-scales, through DVM, SVM or OVM. In terms of DVM, Longhurst *et al.* (1990) explained, "For diel migrant biota to produce a downward vertical flux two conditions must be met. The migration must cross a permanent barrier to physical vertical mixing, and (on balance)...[material]...must be acquired shallower than this barrier for subsequent release deeper". This mechanism is often referred to as 'active transport', or the 'active flux', and it is the quantification of this flux that forms the focus for the present study. As with the passive flux of dissolved material, the active flux has been suggested as another mechanism by which observed shortfalls in the balance between production and export can be made up (e.g. Murray *et al.*, 1989; Steinberg *et al.*, 2000; Hernández-Léon *et al.*, 2001; Palmer & Totterdell, 2001).

Pearre (2003) provided a comprehensive review of past ideas and research regarding the active flux. What must have been one of the earliest mentions in the literature was made by Gran (1929), who suggested that vertically migrating fish should be transporting "nutritive materials" to depth. Other authors suggested that zooplankton

would also play a role (e.g. Redfield *et al.*, 1937; Riley, 1951; Grey, 1956; Wickstead, 1962; Banse, 1964). Perhaps one of the most notable of the earlier proponents for the existence and potential importance of the active flux was Vinogradov (1962), who suggested that the vertical migration behaviour of the interzonal zooplankton ought to be causing a downward transport of material. He suggested a "ladder of migrations", through which the overlapping vertical distributions of migrators could relay material to the depths (also see Mauchline, 1972). Until the late 1980s, however, it appears that consideration of the active flux was confined to the realms of speculation only (see references in Pearre, 2003). This is perhaps because the active flux was deemed by many to be inconsequential in comparison to the passive particle flux. An example of the emergent view, however, came from Angel (1985, 1989a, b) and Bruland *et al.* (1989), who suggested that, particularly in well stratified areas, the active flux may well be of great importance.

A number of earlier investigations had begun to infer the magnitude and importance of zooplankton-mediated export fluxes (e.g. Tseytlin, 1982; Wiebe *et al.*, 1979), but it was not until 1988 that the first fully-focused attempt to quantify the active flux in the marine environment was made (Longhurst & Harrison, 1988). This study appeared to have been inspired, at least in part, by the 'biomanipulation' studies of Kitchell *et al.* (1979) in lake ecosystems. Further investigations soon followed, including those of Longhurst *et al.* (1989, 1990), Dagg *et al.* (1989), Morales *et al.* (1993), Dam *et al.* (1993, 1995) and Atkinson *et al.* (1996). Each of these studies reinforced the potential importance of the active flux caused by the DVM of interzonal migrants. For example, Longhurst *et al.* (1990) estimated an annual global respiratory carbon (DIC) flux of up to 0.27 Pg C, which is 7.7 % of Sundquist's global estimate for the passive POC flux. As for the effects of migrations over longer time scales, Longhurst & Williams (1992)

suggested that SVM, at least for *Calanus* in the North Atlantic, was not an important contributor to the export flux. Bradford-Grieve *et al.* (2001), however, showed that the SVM/OVM of *Neocalanus tonsus* in the Southern Ocean had the potential to remove far more significant amounts of carbon to the ocean's interior.

Up until 1997, no attempts had been made to quantify the overall active flux of carbon and nitrogen caused by DVM. Thus far the focus had been on individual parts of the jigsaw (specifically, the excretion of DIN and respiration of DIC at depth) using a mixture of net-derived measurements of size-fractionated zooplankton and micronekton biomass, and *in vitro* measurements of various metabolic rate processes. The *in vitro* measurements, in particular, are likely to have suffered from inaccuracies due to the effect of laboratory conditions on planktonic animals (Ikeda, 1977), and it is likely that such problems would have reflected on the accuracy of the active flux estimates. In order to address this issue, Hays *et al.* (1997a) devised a simple *in situ* technique (referred to throughout this thesis as "ZOOFLUX", see section 2.1), which, they proposed, should enable the quantification of the overall active fluxes of carbon and nitrogen in any given area of the ocean. Following a simple laboratory experiment that suggested their technique was "ripe for application" (Hays *et al.*, 1997a), they were able to apply this approach in the field with encouraging results (Hays *et al.*, 1997b, 2001b).

There have been a number of publications concerning the active flux since this time (e.g. Le Borgne & Rodier, 1997; Zhang & Dam, 1997; Morales, 1999; Steinberg *et al.*, 2000, 2002; Al-Mutairi & Landry, 2001; Bradford-Grieve *et al.*, 2001; Hernández-Léon *et al.*, 2001; Hidaka *et al.*, 2001; Zeldis, 2001; Schnetzer & Steinberg, 2002a), although none have employed the ZOOFLUX approach. From these studies, nearly all of the aspects of the zooplankton-mediated active fluxes of carbon and nitrogen have now been investigated, specifically those caused by respiration (DIC), excretion (DIN, DOC,

DON), defaecation (POC, PON), and mortality (POC) at depth. Only the production of exuviae (moulting) and eggs (spawning) at depth has remained largely unaddressed. While both mechanisms can represent major avenues for material release into the environment in certain cases, they will only contribute to the active flux if they are performed at depth: reference may be made to Kuenzler (1969), Hosie & Ritz (1983) and Steinberg *et al.* (2000) for discussions on moulting, and Nicol *et al.* (1995) for an example of the potential importance of egg production. The numerous studies to date have drawn five main conclusions: (1) interzonal migrants can significantly enhance oceanic export fluxes via their DVM behaviour, (2) the relative importance of the active flux is highly dependent on the biomass of the migrating community, (3) the relative importance of the active versus the passive flux increases with increasing depth, (4) interzonal migrants may provide a steady source of nutrients to deep-sea microbial communities, and (5) we should be including the active flux caused by DVM within considerations of the cycling of biogeochemically important elements.

2

AN *IN SITU* TECHNIQUE FOR MEASURING THE ACTIVE FLUX OF CARBON AND NITROGEN: RATIONALE AND PRACTICAL CONSIDERATIONS

2.1 The ZOOFLUX technique

Recently, Hays *et al.* (1997a) suggested a simple way in which the active fluxes of carbon and nitrogen caused by interzonal NDVM might be measured *in situ*. For convenience, this technique will be referred to as "ZOOFLUX". Having received only one field trial to date (Hays *et al.*, 1997b, 2001b), the purpose of this study was to apply ZOOFLUX further in the field and, if applicable, to develop and refine the methodology so that it might become an established and widely used oceanographic field technique.

The central tenet of ZOOFLUX is that zooplankton undertaking interzonal NDVM will incur a net gain of carbon and nitrogen while actively feeding in the food-rich surface layer of the ocean at night (Figure 2.1). Furthermore, as they descend to depth through the pycnocline at dawn, food levels, and therefore feeding, are either non-existent or much reduced, and they will incur a net loss of this carbon and nitrogen via the continuing metabolic processes of respiration, excretion and defaecation. Further losses may also occur via moulting, egg production, and mortality (via either senescence or predation). The difference between the carbon and nitrogen weight of a full animal just as it crosses from the surface layer to the deep at dawn, and an emptier one as it crosses from the deep to the surface layer at dusk, will therefore represent the total amount of carbon and nitrogen lost in the deep layer. An analogy would be this: by counting the amount of money in a person's pocket as they enter a shop, and again when they leave, one can infer the amount of money that they had spent while inside.

Measurement of the dawn-dusk difference in both the carbon and nitrogen weight of interzonal migrants may therefore provide valid estimates for the total active flux of these elements. However, since the destructive nature of sampling precludes the ability to follow the same individual over time, separate individuals must be sampled at different stages of the diel cycle. This means that the measured dawn-dusk difference

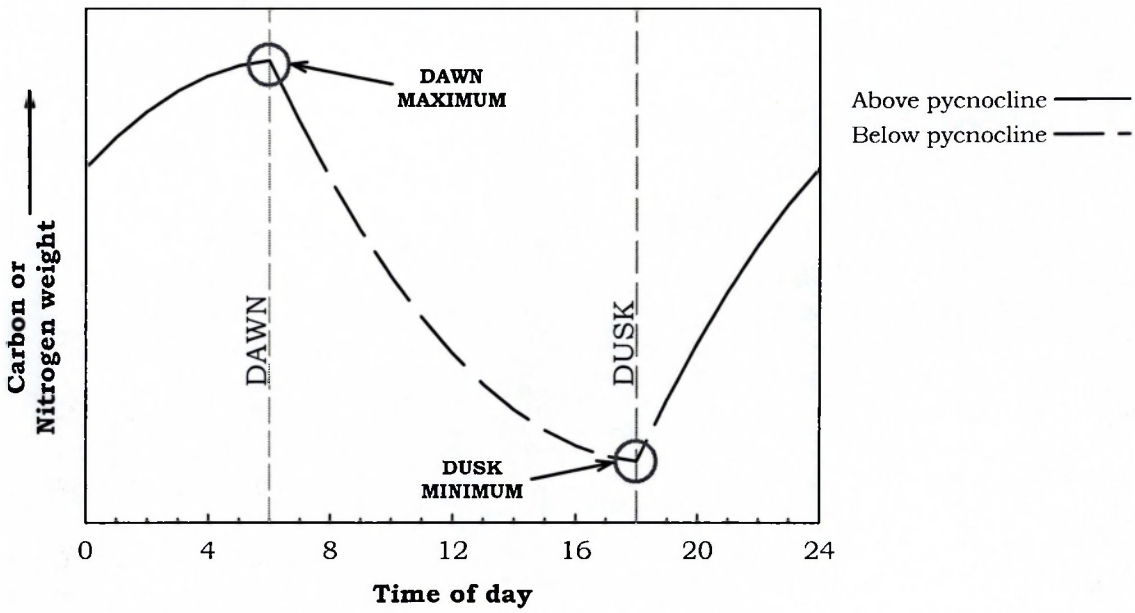

Figure 2.1 A graphical representation of the diel changes in carbon or nitrogen weight expected in a zooplankton interzonal vertical migrant that spends the hours of darkness feeding in the surface mixed layer, and the daylight hours taking refuge beneath the pycnocline in the relatively foodless depths.

actually represents the average diel change in the population. The mean dawn-dusk difference between individuals (μg C or N $ind.^{-1}$ d^{-1}), when multiplied by the number of individuals migrating through the pycnocline (ind. m^{-2} d^{-1}), provides an estimate for the active flux of carbon and nitrogen at any given sampling site (μg C or N m^{-2} d^{-1}). It is useful to compare these assessments with concurrent measurements of primary production and passive flux in order to assess the relative importance of the active flux at this particular place and time.

2.2 The practicalities of field-sampling

"I knew that boats ran all night, but somehow I had never happened to reflect that somebody had to get up out of a warm bed to run them".

Mark Twain, "Life on the Mississippi" (1883)

A major advantage of ZOOFLUX is that it relies on well-established and relatively simple field and laboratory techniques. However, some of these techniques, while simple, require specialist equipment that may not always be readily available.

2.2.1 Obtaining samples and measuring the dawn-dusk difference

The timings of dawn and dusk for a given date and location may be predicted prior to a sampling cruise by consulting nautical almanacs or certain web pages (e.g. URL: http://aa.usno.navy.mil/data/). This gives at least an estimate of when interzonal migrants are likely to be ascending and descending through the pycnocline, so that the timing of specimen collections can be planned. The presence or absence of a pycnocline can be confirmed from conductivity-temperature-depth (CTD) casts. Interzonal zooplankton migrants such as copepods and krill may be collected with simple plankton nets fished at depths appropriate to the depth of the animals at that particular time. Around dawn and dusk, one might expect migrants performing interzonal NDVM to be near to the pycnocline. To avoid the inclusion of zooplankton from shallower depths, opening/closing nets are available which allow animals to be collected from discrete depth intervals. Once collected, the carbon and nitrogen weight of the target animals may be measured in the laboratory via combustion in an elemental (CHN) analyser.

2.2.2 Gaining an understanding of the zooplankton community

To obtain samples suitable for the application of ZOOFLUX, one therefore needs to know the identity of the significant interzonal migrants at the study site in question, and to have at least a rudimentary understanding of their DVM behaviour. It is important to make net tows as close as possible to the times at which migrants ascend and descend through the pycnocline, so that any observed carbon- and nitrogen-weight differences

will represent the most accurate assessments of carbon and nitrogen loss at depth. Depending on the sampling location and the time of year, knowledge of the zooplankton environment will vary considerably. In a less studied area, it may be necessary first to make exploratory net tows at different depths and at different times over the diel cycle in order to assess the species present and their movements. Simple counts of individuals within depth-discrete tows can provide a certain amount of information on the vertical movements of the community, while the analysis of individual-based "tracers", such as body length, gut fullness and carbon or nitrogen weight, may provide insights into the movement of individuals (see Pearre, 1979a, in press). Acoustic instruments such as the acoustic Doppler current profiler (ADCP) are capable of providing near real-time information on the distribution and vertical velocity of zooplankton, and represent an additional tool for the study of DVM behaviour (e.g. Pleuddemann & Pinkel, 1989; Tarling *et al.*, 2002).

2.2.3 Measuring primary production and passive flux

Primary production is typically estimated by measuring the uptake of radioactive ^{14}C into phytoplankton cells incubated *in situ* in polycarbonate bottles, and passive particle flux by suspending sediment traps at predetermined depths in the water column (see e.g. Steinberg *et al.*, 2001). More sensitive sampling techniques are required when estimating the passive flux of dissolved material, but it is nevertheless a measurable process. For instance, dissolved organic carbon (DOC) in depth-discrete water samples may be measured by the High Temperature Combustion (HTC) method (see Carlson *et al.*, 1994) to provide vertical DOC profiles of the water column. This determines a gradient that allows the direction and rate of diffusion to be predicted.

2.3 Data analysis

"There is something fascinating about science. One gets such wholesale returns of conjecture out of such a trifling investment of fact".

Mark Twain, "Life on the Mississippi" (1883).

Studying the relationships between the 'biometric' parameters of individuals can help elucidate the ecology of the population. Biometric parameters include body length and width, gut fullness, and body weight (dry, carbon, and nitrogen). Furthermore, the ZOOFLUX technique relies on the detection of changes in body weight over time to estimate the net amount of material that migrants release while at depth. Data analysis is therefore an important aspect to the present study. The ecological significance of the various biometric parameters measured in the present study is introduced below, and descriptions provided of the statistical analyses performed (i.e. regression, ANOVA, and ANCOVA). This section concludes with a description of the ways in which the success of any field application of ZOOFLUX can be quantified in statistical terms.

2.3.1 The ecological significance of various biometric parameters

Body length

Length is a standard measure of 'size' in animals, and one that is easily measured with a relatively high level of precision. Its primary function during the present study was to allow the carbon and nitrogen weight of individuals to be 'normalised' to a uniform body size (see section 2.3.6 below). However, this parameter is also useful to know within the context of an active-flux study for two reasons. Firstly, the magnitude of the export flux (both passive and active) is likely to be influenced by the size of the individuals within the zooplankton community (Michaels & Silver, 1988). Larger individuals may enhance the passive flux via the production of larger, faster-sinking

faecal pellets (e.g. Tseytlin, 1999), moults and carcasses. Larger individuals may also enhance the active flux by carrying more material per individual across the pycnocline, and by releasing more metabolites per unit time at depth. Secondly, size may relate to individual variability in DVM behaviour. As Hays *et al.* (1994) showed, the size of an individual copepod, in conjunction with its colour and morphology, may relate to its susceptibility to visually orienting predators. Larger, more visible individuals may need to hide out at depth for a greater portion of the diel cycle, allowing more metabolites to be released and thereby enhancing the active flux. Also, as Hays *et al.* (1998, 2001a) have shown, the body condition of animals may dictate their levels of foraging. An example given in Hays *et al.* (2001a) was that of nocturnal bats: when body condition was poor, individuals needed to extend foraging into the daylight hours, despite the increased risk of predation from raptorial birds. For migrant zooplankton, a better body condition might mean that they may not need to make the risky move to the surface to feed as often, and this will have implications for the active flux. Since a standard index of body condition in animals is their mass to length ratio (e.g. Cavallini, 1996), it is apparent that length measurements are also useful in this context.

Gut fullness

The presence of food material in the guts may be used as an indicator of recent feeding. In conjunction with information on the vertical distribution of migrants and their food, and a knowledge of their gut-filling time (GFT) and gut-passage time (GPT), this may help to decipher individual DVM movements and the most likely fluxes of food-derived carbon and nitrogen. It is also useful to know the amount of material in the guts relative to the overall mass of the animal, in order to assess the relative importance of defaecation as a process by which material is released into the environment.

Dry weight

Measuring the carbon and nitrogen weight of zooplankton is both more expensive and more complex than measuring dry weight. It would therefore be useful if dry weight could be used as a reliable proxy for carbon and/or nitrogen weight. The suitability of this parameter as a proxy will vary with the site and season, and depends on the strength of the relationship between dry weight and either carbon or nitrogen in each case.

Carbon and nitrogen weight

Measurements of the carbon and nitrogen weight of zooplankton vertical migrants are central to the application of ZOOFLUX. While also relating to size, as with length, volume and dry weight, the relative amounts of carbon and nitrogen within individuals may vary according to the proportion of lipids (carbon-based) to proteins (nitrogen-based). Since these may change over a variety of timescales, a cautionary note must be attached when attempting to use carbon or nitrogen weight as a measure of size. However, the carbon to nitrogen ratio (C:N) is a useful indicator of the relative amounts of lipids and proteins, which may, in turn, highlight information about a given individual (e.g. body condition, or the storage of lipids before overwintering). As with gut fullness, such individual-based information may prove to be useful in deciphering the movements of individuals within a migrant population. C:N is most accurately expressed in terms of atoms, and is calculated in the following way:

$$\mathrm{C:N\,(atoms)} = \frac{\text{Carbon weight} / 12}{\text{Nitrogen weight} / 14}$$

Equation 2.1

2.3.2 Data quality control

Before any statistical analysis, it is always useful to assess the quality, and hence reliability, of the data. This can be expressed in terms of accuracy, calculated as a percentage error according to the equation:

$$\% \text{ error} = \pm x/v \times 100$$

Equation 2.2

where x is the precision of the measuring equipment used, and v is the value of the measurement made using this equipment. 'Precision' and 'accuracy' are used here *sensu* Zar (1999), who wrote: "Accuracy is the nearness of a measurement to the actual value of the variable being measured. Precision is not a synonymous term, but refers to the closeness to each other of repeated measurements of the same quantity". In the present study, only those data values with an accuracy of ±5 % or better were used for analysis.

2.3.3 Variability

As Zar (1999) also advised, "it is very important to provide the reader of a research paper with some information concerning the variability of the data reported". Variability in a biological dataset will be due to both methodological errors and 'true' biological variability. The decision as to which source is the most influential for any given dataset may be subjective, based on interpretations from patterns that may be apparent in the measures of central tendency and dispersion.

Since the desire in the present study was to describe variability within the population (as opposed to the precision of the estimate of the population mean), this information was presented in terms of the sample mean and the sample standard deviation (SD). The sample mean is a measure of central tendency, and is calculated as:

$$\text{Mean} = \frac{\sum X_i}{n}$$

Equation 2.3

The statistic, SD, is a measure of dispersion, and is calculated as:

$$\text{SD} = \sqrt{\frac{\sum X_i^2 - \frac{(\sum X_i)^2}{n}}{n-1}}$$

Equation 2.4

SD has a magnitude that is dependent upon the magnitude of the data, so it cannot be used directly to compare the variability of two sets of data that have either different magnitudes or different units (for example, one cannot compare variability in mouse ears with that in elephant ears). However, direct comparisons can be made between measures of variability when expressed as the coefficient of variation (V). The statistic, V, is a measure of relative dispersion and therefore has no units. Typically expressed as a percentage, it is calculated as:

$$V = \frac{\text{SD}}{\text{Mean}} \times 100$$

Equation 2.5

2.3.4 Simple linear regression: analysing the relationships between parameters

Analysis of the relationships between the biometric measurements made on an individual can yield important ecological information. Statistical techniques that consider the relationships between two variables include simple linear regression and simple linear correlation. Regression is used when the magnitude of one of the variables

(the dependent variable) is a function of the magnitude of the second variable (the independent variable), but not *vice versa*. Correlation is used when it is unreasonable to consider there to be a dependent and an independent variable. The term 'simple' refers to the fact that only two variables are being considered, while the term 'linear' refers to the additive nature of the equation describing the relationship.

In this study, linear regression as opposed to correlation was used to describe the relationships between measurements. In regression analysis, the independent variable (X_i) is plotted against the dependent variable (Y_i). The equation describing the line of 'best fit' through the data-points is the one that minimises the sum of the squares of the deviation of each Y_i value from this line. This is known as the 'least squares' method*. An r^2 value, or 'coefficient of determination', describes the 'goodness' of this fit (0 = no relationship, 1 = perfect fit through all points). A probability value (ANOVA: P), typically at the 5 % significance level ($\alpha = 0.05$, see section 2.3.5 below), represents the likelihood that the slope of this line (b) is zero, i.e. that there is no relationship between the variables. While Zar (1999) provided equations for manually calculating the various regression parameters, simple linear regression is quickly and easily performed on two columns of data by a variety of statistical software packages.

As the name suggests, linear regression is applied to data that exhibit a linear relationship. However, many morphological and physiological variables (Y_i values, e.g. dry weight, carbon weight) scale with body size (X_i values, e.g. length) according to the equation:

$$Y_i = aX_i^b$$

Equation 2.6

* Another method is the 'least absolute deviation' method, or LAD.

This relationship is a model of 'exponential growth' and, as such, is non-linear with regard to its parameters. However, by taking the logarithm* of each side of the equation, one acquires a model that is linear (i.e. additive) with regard to its parameters:

$$\log Y_i = \log a + b \log X_i$$

Equation 2.7

Therefore, the log-transformation of two variables that exhibit a non-linear relationship often enables this relationship to be described as a straight line (e.g. Bottrell *et al.*, 1976; McCauley, 1984; Bird & Prairie, 1985). As Zar (1999) wrote, "often, logarithmic or other transformations of the values of Y and/or X will result in a straight line relationship amenable to linear regression techniques". However, he also warned that care must be taken so that the assumption of homogeneity of variance (one of five assumptions in regression analysis: see Zar, 1999, p.332) is not violated following transformation.

2.3.5 ANOVA: investigating temporal differences between parameters

Temporal differences in the length, gut fullness and body weight of individuals can be investigated by comparing the values obtained from samples collected at different times. A 'time-point' (or 'group') in the present study refers to either a period of time during the diel cycle, or a particular sampling month. Such comparisons can be made using analysis of variance (ANOVA), a statistical tool that takes into account the variances in the data when comparing group means. As Sokal & Rohlf (1995) explained, ANOVA

* Logarithms (often shortened to "log") were devised by John Napier in 1614. The word derives from Greek: *logos* ratio, reckoning + *arithmos* number. If $a^x = M$, then the log of M to the base a (i.e. $\log_a M$) will be x.

"is a tool that can provide an insight into the nature of variation of natural events, into Nature in short". However, they also warned that ANOVA "may create constructions of nature in the mind of the scientist that give rise to misleading or unproductive conclusions". It is apparent that such a statistical procedure is of great use when attempting to understand measurements of the natural world, but that caution must be exercised when inferring biological from statistical 'significance'. Indeed, concerning the use of statistics in general, Schmidt-Nielsen (1984) wrote, "we must realise that the proper statistics are no more than a description of the numbers at hand".

In the present study, differences between measurements made at different times were tested for using 'one-way', or 'single factor', ANOVA (the 'factor' here being 'time'). Before this test can be performed, two assumptions must be met: (1) that the data from each time-point come from a 'normally' distributed population; (2) that the variance is equal at each time-point. Checks that these assumptions were met were carried out before running the ANOVA test: a Kolmogorov-Smirnov test was employed to test for a normally distributed population, while a Levene's test for the equality (or homogeneity) of variance checked the variability about the group means. If the probability (P) value computed by these tests was >0.01 (i.e. if the probability of being wrong in concluding a non-normal distribution or unequal variance was >1 %), then the test passed and ANOVA could be performed.

Where a test of the difference between only two time-points ($k = 2$) is desired, either a two-sample t test or a one-way ANOVA will be appropriate. Both of these will yield identical conclusions. For the sake of consistency, one-way ANOVA was therefore used throughout the present study. Significance limits for the ANOVA tests performed here were set at the standard levels ($\alpha = 0.05$, $\beta = 0.1$). The alpha value (α) represents the acceptable margin of error (5 % in this case) for concluding that there are 'significant'

differences between the time-point means: if the probability (P) of being wrong when saying that the differences are significant is ≤5 %, then these differences are accepted as 'real'. This P-value is also known as the probability of making a "Type I" statistical error (i.e. saying that there is a significant difference when really there isn't), whose acceptable limit is α: a Type I error is said to have occurred if $P > \alpha$. In those instances when no significant differences are found between the time-point means (i.e. ANOVA: $P > 0.05$), the beta value (β) represents the acceptable margin of error (10 % in this case) that this conclusion is wrong. This β-value is also known as the probability of making a "Type II" statistical error (i.e. saying that there is no significant difference when really there is): a Type II error is said to have occurred if ANOVA: $P > 0.05$ and $\beta > 0.1$. Often, the reciprocal of β (i.e. $1-\beta$) is referred to as the 'power' of an ANOVA test. The ANOVA test statistic, F, at $\alpha = 0.05$, is typically presented as $F_{(k-1),(N-k)}$, where N is the total number of measurements in the k time-point groups. When a significant difference between the k time-point means is found (ANOVA: $P \leq 0.05$), a Tukey multiple comparison test is performed to find out which means differ significantly from which others (Tukey: $P \leq 0.05$), and by how much.

When the data are non-normally distributed (Kolmogorov-Smirnov: $P \leq 0.01$) and/or exhibit unequal variances (heteroscedasticity) (Levene's: $P \leq 0.01$), a nonparametric Kruskal-Wallis test (or 'ANOVA by ranks') is performed. The Kruskal-Wallis test is identical to the parametric one-way ANOVA, but is performed on the ranks of the data (values are ordered from highest to lowest). It is known as a nonparametric test since it is now no longer concerned with specific parameters (such as the mean in ANOVA) but only the distribution of the variates. Because of this, the differences between time-point medians, as opposed to means, are tested during non-parametric situations (the median is the middle measurement in an ordered set of data). As with parametric ANOVA,

when $k = 2$, the conclusions of a Kruskal-Wallis test will be identical to those of its nonparametric t test counterparts, either the Mann-Whitney U-test or the Wilcoxon two-sample test. Again for the sake of consistency, the Kruskal-Wallis test was used throughout the present study. The significance limits were set at the same values as for ANOVA, and the test statistic, H, at $\alpha = 0.05$, is presented as H_{DF}, where DF (degrees of freedom) = $k - 1$. Where a significant difference between the time-point medians was found (Kruskal-Wallis: $P \leq 0.05$), a Dunn's multiple comparison test was performed to find out which medians differed significantly from which others (Dunn's: $P \leq 0.05$), and by how much.

2.3.6 ANCOVA: mitigating the effects of size-induced individual variability

Any individual variability found in the carbon and nitrogen weight of members of a sampled population will be due to a combination of methodological factors, 'true' biological variability (i.e. differences due to biological factors), and variability due to body 'size'. For example, for a given species of interzonal migrant, if one individual collected at dawn was found to contain more carbon than another collected at dusk, one might like to conclude that this difference was due to a 'biological' factor such as reduced feeding and continuing metabolism during the daytime. However, one might equally attribute this difference to the individual at dusk being smaller, in which case there is no way of knowing whether, in this model of average change, a biological loss of carbon was in fact occurring. It is evident that this size-induced variation must be removed before true biological changes in carbon and nitrogen weight can be identified. One way to reduce the amount of size-induced variation might be to sort individuals by developmental stage. Where this is impractical, or where intra-stage variability in size is still high, further mitigation may be achieved by standardising (normalising) the data to

a uniform size.

Heeding the advice of Packard & Boardman (1999), size normalisation was carried out using a procedure known as analysis of covariance, or ANCOVA. As they explained, ANCOVA "is not nearly as intimidating as its name might imply. ANCOVA is a blend of two statistical procedures with which virtually all physiologists are familiar: regression and the ANOVA. Regression removes effects of body size from the variable of interest and ANOVA then provides a sensitive test of the adjusted data". Both regression and ANOVA have been introduced above.

One begins the ANCOVA procedure by quantifying the relationship between the measure of size (X_i values) and the variable to be size-normalised (Y_i values) using simple linear (least squares) regression. This relationship is described by the equation:

$$Y_i = a + bX_i$$

Equation 2.8

The regression coefficient, or slope (b), is then used to calculate the size-normalised value ($\hat{Y}$) at a defined size (X_M) via the equation:

$$\hat{Y} = Y_i + b(X_M - X_i)$$

Equation 2.9

Carbon and nitrogen weights adjusted in this way therefore provide a better measure of true inter-individual variability without the inclusion of size-induced variation.

2.3.7 Calculating the active flux according to ZOOFLUX

In those instances where a significant dawn-dusk difference in the mean carbon or

nitrogen weight of individuals was found (ANOVA: $P \leq 0.05$), one could say that an active flux had occurred (note that this could be either downward or upward). This dawn-dusk difference (μg C or N $ind.^{-1}$ d^{-1}), when multiplied by the estimated number of animals migrating across the pycnocline (ind. m^{-2} d^{-1}), will therefore provide a depth-specific value for the active flux (μg C or N m^{-2} d^{-1}). This value may also be expressed as a percentage of depth-integrated primary production or passive particle flux at the appropriate depth, where such data are available.

2.3.8 Quantifying the statistical success of ZOOFLUX

Hays *et al.* (1997a) pointed out that the ability to detect a significant (ANOVA: $P \leq 0.05$) dawn-dusk difference in carbon or nitrogen weight (and hence, the success of ZOOFLUX) depends on two factors: (1) the true magnitude of the diel change, and (2) the inter-individual variability (V) in carbon or nitrogen weight. They performed a simple model (based on a t test), which showed that a relatively small dawn-dusk difference (<5 %), accompanied by relatively high V (>10 %), will result in a variety of statistical problems that mean the dawn-dusk difference cannot be reliably quantified. We should therefore like to quantify these statistical problems in order to assess the statistical 'success' of ZOOFLUX when applied in the field.

Following the measurement of the mean carbon and nitrogen weight of individuals at different times during the diel cycle, and the removal of size-induced variation using ANCOVA, there will exist a measure of the magnitude of the dawn-dusk difference in conjunction with a good assessment of the degree of the true biological variability inherent in these parameters. ANOVA yields two sources of variation in the data, namely within-groups ('error' or 'residual') and between- (or among-) groups ('groups'). This error and groups variation is expressed initially in terms of the sum of

the squares of the deviations from the means (sum of squares, SS, or 'variance'). Dividing the error SS or the groups SS by their respective degrees of freedom, or DF (error DF = $N - k$, where N = the total number of samples; groups DF = k - 1), results in a variance referred to as the mean square (MS). The error MS and groups MS therefore take into account the sample size, and are the best single measures of variability in the data.

Using these measures of variability, the statistical 'success' of ZOOFLUX can be expressed in three ways:

1. *The probability of committing a Type II statistical error.* In those instances where the dawn-dusk difference is not deemed to be significant (ANOVA: P >0.05), one can determine the probability ($\beta \times 100$) that a Type II error had been made, i.e. that a true difference was being masked by either the number of samples being too low and/or the biological variability being too high. Let us consider a one-way ANOVA involving $k = 2$ groups (i.e. dawn and dusk) which has been performed at the $\alpha = 0.05$ significance level, with n samples per group that did not detect a significant dawn-dusk difference in carbon or nitrogen (P >0.05). Knowing the error MS and groups MS, it is possible to calculate the power of this test ($1-\beta$). That is to say, with knowledge of the variability in the data and the number of samples collected (both are incorporated in the MS terms), it is possible to quantify the probability ($\beta \times 100$) that the conclusion of a non-significant difference (ANOVA: P >0.05) is wrong. This probability is calculated by computing a quantity called ϕ (Greek lowercase 'phi') from the equation:

$$\phi = \sqrt{\frac{(k-1)\,(groups\ MS - error\ MS)}{k(error\ MS)}}$$

Equation 2.10

The power of the test (1-β) is calculated from ϕ by consulting standard curves (see e.g. Appendix Fig. B.1 in Zar, 1999) at the appropriate degrees of freedom (groups DF (v_1) = $k-1$; error DF (v_2) = $N-k$).

2. *The minimum number of samples required.* For the level of variability observed in the field (error MS), calculations are made to determine the minimum number of samples (n_{min}) that would have been required to conclude that the observed dawn-dusk difference (δ) was significant (ANOVA: P $\leq$0.05, with a power of 1-β = 0.9). With previous experience in the field, it is possible to gauge whether this is an achievable number of samples to collect. This is calculated from the following equation:

$$\phi = \sqrt{\frac{n\delta^2}{2(k)(error\ MS)}}$$

Equation 2.11

Since this equation does not have the desired parameter, n, as the subject, the calculations need to be performed in an iterative manner. That is to say, one begins with an initial guess as to the value of n. The value of ϕ thereby calculated is then used to calculate 1-β, again by consulting standard curves (see e.g. Appendix Fig. B.1 in Zar, 1999) at the appropriate degrees of freedom (groups DF (v_1) = $k-1$; error DF (v_2) = $N-k$). It is then simply a case of refining the estimates of n until the nearest point to the desired power (in this case 1-β = 0.9) is reached.

3. *The minimum detectable dawn-dusk difference.* For the level of variability (error MS) observed in the field, and the number of samples that were collected (n), calculations are made to determine the minimum dawn-dusk difference (δ_{min}) that would have been significantly detectable (ANOVA: $P \leq 0.05$, with a power of $1-\beta = 0.9$). From literature values on the likely metabolic rates of the species in question, it is possible to gauge whether this minimum diel change is realistic. By consulting standard curves (see e.g. Appendix Fig. B.1 in Zar, 1999) at $\alpha = 0.05$, groups DF (ν_1) = 1, error DF (ν_2) = $N - k$, and $1-\beta = 0.9$, one can obtain a value for ϕ to enter into the following equation (which is actually a rearrangement of Equation 2.11):

$$\delta = \sqrt{\frac{2(k)(error\ MS)(\phi^2)}{n}}$$

Equation 2.12

In Equation 2.12, n was entered as the arithmetic mean of the number of samples collected at dawn and at dusk.

3

A CONSIDERATION OF THE ZOOPLANKTON SAMPLING TECHNIQUES USED IN THIS STUDY: NET TOWS AND BIOACOUSTICS

3.1 Introduction

In this investigation of the active flux of carbon and nitrogen in the marine environment, various zooplankton-sampling techniques were employed. Fieldwork involved the deployment of WP-2 and MOCNESS plankton nets for the collection of specimens, and the use of acoustic Doppler current profilers (ADCPs) to describe the vertical and temporal distribution of zooplankton in their natural environment. Specimens collected in the nets were maintained alive for shipboard experiments, frozen for subsequent biometric measurements, or fixed, preserved and counted to estimate species composition and concentration. An appreciation of the nature of these data sets is important in their interpretation.

The use of plankton nets dates back to Dr J. Vaughan Thompson in 1828, who constructed a net to sample crab and barnacle larvae. The simple process of towing a filtering mesh through the water has not changed in essence to the present day: the only improvements have been in our ability to filter discrete portions of the water column, and to make concurrent biological, chemical and physical measurements. While the collection of zooplankton is therefore relatively simple, understanding the data so gathered is an inherently more difficult matter. This is due to a number of errors associated with net tows. These errors can be sub-divided into those incurred during the tow, and those incurred during sample processing and sub-sampling. The use of ADCPs for studying zooplankton has a much shorter history, with the first published study being carried out by Flagg & Smith (1989). The ADCP is technically a more complex measuring device than the standard plankton net, as is the processing and interpretation of the data provided.

This chapter therefore considers the net-tow and acoustic methods used in this study, beginning with a brief introduction to the general constraints inherent in any

zooplankton-sampling programme. Net-tow considerations are discussed in terms of the potential errors incurred during tows, and those incurred during sample processing. Acoustic considerations include the nature of sound energy in water, and the various issues that have arisen through past research. A recent review of these and other zooplankton-related topics has been provided in the ICES Zooplankton Methodology Manual (Harris *et al.*, 2000).

3.2 General constraints of sampling programmes

Any zooplankton-sampling programme will be subject to a variety of constraints that may limit the amount and/or quality of the data obtained. These may be broadly considered as logistical, financial, biological or physical. Logistical and financial constraints will include the distance of the study site from the nearest port or harbour, limits as to the types of vessel and sampling equipment that can or need to be used, the inherent costs of the sampling programme, and the funding and equipment available. Biological constraints when sampling for ZOOFLUX include the behavioural and physiological variability of the target species. Physical constraints include weather conditions that limit the ability to reach and sample at sites of interest. These aspects are considered in more detail with relevance to the present study in section 7.3.1.

3.3 Zooplankton net tows

3.3.1 Potential errors during tows

Plankton nets are designed to collect all the target species in their path, and to allow the volume of water filtered to be either measured or inferred (Atkinson, 1990). There are, however, a variety of reasons as to why these criteria might not be met: net avoidance, escapement through the mesh, contamination of closed nets, and net clogging. As

Sameoto *et al.* (2000) pointed out, these various problems form part of the reason why a huge variety of plankton nets exist to perform what, at first consideration, would appear to be a relatively simple task. These issues were comprehensively addressed between 1964 and 1966 by a joint working group of scientists nominated by the International Council for the Exploration of the Sea (ICES), the Scientific Committee on Oceanic Research (SCOR) and the United Nations Educational, Scientific and Cultural Organisation (Unesco). Their results, presented at the Symposium on the Hydrodynamics of Zooplankton Sampling, held in Sydney in February 1966, are summarised in a Monograph on Oceanographic Methodology (Unesco, 1968). A recent review of the factors influencing mesozooplankton samples is provided in Sameoto *et al.* (2000).

Net avoidance

The active swimming of zooplankton (a group which, by definition, is envisaged to drift passively in the water column) out of the path of an approaching net is generally thought to be the most serious of the potential net sampling errors, and few solutions exist to combat this problem effectively (Sameoto *et al.*, 2000). According to Tarling (1995), anecdotal observations of net avoidance by zooplankton are common. A dramatic example given was that of Mackintosh (1934), who described how krill in the Southern Ocean "could clearly be seen to leap backwards out of the way of the approaching net". Just as common, it would appear, are observations that are more empirical. Clutter & Anraku (1968) provided a comprehensive reference list of the "overwhelming" evidence for net avoidance in zooplankton and micronekton, and discussed this phenomenon in terms of the response of individuals to physical disturbances caused by the passage of the net. In essence, an individual will be more

successful in avoiding a net if it can feel or see it in time, and if it can move out of its path quickly enough. Avoidance may therefore be reduced when using larger nets (e.g. McGowan & Fraundorf, 1966), when light levels are reduced (e.g. Fleminger & Clutter, 1965; Sameoto, 1983), when towing speeds are increased (e.g. Barkley, 1964, 1972), when warps, bridles and other obstructions are removed from in front of the net, and/or when the net is of an inconspicuous colour (e.g. Wiebe *et al.*, 1982).

Mesh selection (retention vs. escapement)

One might expect that zooplankton entering a towed plankton net will either be retained within the net if they are larger than the mesh size, or will escape through the mesh if smaller. In his assessment of the loss of organisms through the meshes of towed nets, Vannucci (1968) introduced the term "mesh selection" to describe "the capacity of the net to select individual organisms from the population in the water that has passed through the mouth of the net". In this assessment, however, it was also explained that retention versus escapement is not solely a function of an individual's size in relation to that of the mesh opening. Other factors to consider include an individual's shape (including texture, spines and other protrusions), plasticity (which may be considerable: see Saville, 1958) and behaviour, as well as the nature and weave of the mesh material, its propensity for clogging, and the age of the net. Indeed, as Fraser (1968) wrote, "How much easier it would be to sample plankton if it consisted only of smooth spherical balls!"

As well as individuals smaller than the mesh size passing freely through the mesh opening, further 'passive escapement' may occur when water pressure associated with the flow forces an individual through the mesh. As Bernhard *et al.* (1973) demonstrated, complete retention will only occur when the mesh diameter is 75 % of the width of the

organism. Similar results were obtained by Nichols & Thompson (1991), whose mathematical model suggested 95 % retention of copepods at a mesh size of 75 % of the carapace width. In addition, 'active escapement' may occur when the behavioural response of an individual, which may vary between species and developmental stages, enables it to squeeze through the mesh.

Contamination of closed nets

As Atkinson (1990) mentioned, errors may arise in the interpretation of depth-discrete samples if organisms from shallower depths are somehow able to find their way into the net while closed. This will be a function of the net design in all cases.

Filtration efficiency (net clogging)

Variation in the "straining capacity", or filtration efficiency, of a plankton net has been recognised as a "fundamental" potential source of error for quite some time (Kofoid, 1897). Filtration efficiency is a function of net design, namely the net shape, mesh size, mesh area, netting porosity (the open area fraction of the mesh area), filtering area and the mesh area to mouth opening ratio (Sameoto *et al.*, 2000). The complexities of the interaction between net and water were comprehensively reviewed by Tranter & Smith (1968), who discussed the theoretical basis and practical consequences for variations in filtration efficiency, net design, and net availability. Smith & Clutter (1965) considered clogging to be a serious issue when filtration efficiency drops below 85 %. They found that clogging rate is affected by the particulate composition of the water column, the mesh size (smaller meshes clog faster), the ratio of filtering area to mouth area (the smaller the ratio, the faster the clogging), and the form of the net (cylinder cones and cylinders are the most efficient). On one hand, the effects of progressive clogging of the

net during a tow may act to reduce the catch, firstly by increasing the extrusion of organisms through the mesh as the pressure increases, and secondly by the creation of a bow wave ahead of the net which may enhance net avoidance. On the other hand, the reduction in the effective mesh size over time may also act to retain progressively smaller and smaller organisms.

As Sameoto *et al.* (2000) discussed, studies of filtration efficiency have led to the formulation of two equations that aid in the design and choice of plankton nets:

$$\mathrm{Log}_{10}(R) = 0.38\left(\mathrm{Log}_{10}\frac{V}{A}\right) - 0.17 \quad \text{Green Water}$$

Equation 3.1

$$\mathrm{Log}_{10}(R) = 0.37\left(\mathrm{Log}_{10}\frac{V}{A}\right) - 0.49 \quad \text{Blue Water}$$

Equation 3.2

where R = filtering area/mouth area, A = mouth area (m^2), and V = volume of water to be filtered (m^3). Given that the filtering area of a mesh is determined as the product of the total area of mesh forming the net (α) and its porosity (β, available from the manufacturers), these equations allow one to choose the appropriate mouth- and mesh-size of a net for any given application.

Spatial and temporal variability (patchiness)

Plankton exhibit "aggregated, patchy distributions of abundance" in both space and time in their natural environment (Haury *et al.*, 1978). As Steele (1978a) wrote, "for the problems of sampling this environment, we have to take account of these different dimensions". That is to say, variability in catch quantity and composition cannot

necessarily be attributed to equipment-related errors alone. Haury *et al.* (1978) summarised the problem: "because we assume our samples represent a larger universe, patchiness strongly affects our efforts to obtain estimates of the abundance of organisms and our ability to detect significant spatial and temporal changes in abundance. It is therefore of great importance that we understand its nature, causes, and effects". A detailed treatment of variability in the plankton, and in particular their spatial pattern, can be found in Steele (1978b).

3.3.2 Potential errors during sample processing

Unfortunately, the potential for sampling errors does not end once a net has been retrieved from the water. Quantitative work begins with the recovery of the sample from the net, and even this is subject to the nature of the sampling programme and the net system used. The way in which organisms are rinsed into the cod end may vary with net type and sampling protocol. For example, closing nets such as the WP-2 net (Unesco, 1968) should be rinsed only at the lower part after stratified sampling (Sameoto *et al.*, 2000), while some investigators do not rinse at all if organisms are required for live work. Following retrieval from the net, samples are then subject to errors depending on whether they are used for live work, or whether they are preserved for identification and/or enumeration.

Processing errors with live samples

Any organism removed from its natural environment for scientific observation will be subject to varying degrees of stress. This may in turn affect how accurately the data obtained will reflect the true nature of the biological parameter under investigation. One needs to be aware that organisms required for live work will have been stressed during

both capture and handling, and that steps should be taken to reduce this stress to a minimum. This might involve making shorter tows with coarser meshes, for example, or leaving experimental animals sufficient time to 'acclimatise' to their laboratory environment before making any observations.

Sub-sampling errors with preserved samples

When plankton samples are required for the identification and enumeration of species in a defined volume of the water column, they are fixed and preserved (see Unesco, 1976, for a full discussion of the various techniques) and subsequently analysed in the laboratory. Ideally, all of the target organisms in the sample should be counted (Venrick, 1971). In many cases, however, sub-sampling is necessary when a sample contains more organisms than is reasonable or affordable to count (Horwood & Driver, 1976). From a number of comparative experiments, Tarling (1995) suggested that counting ~100 individuals of each target organism will be adequate, "for beyond this the increase in accuracy is not enough to warrant the extra effort required". An often-held assumption that errors arising from sub-sampling are small, compared to the actual variation inherent among samples, has been challenged by a number of authors (e.g. Dahiya, 1980; Van Guelpen *et al.*, 1982; Griffiths *et al.*, 1984).

Two pieces of equipment commonly used for obtaining known proportions (aliquots) of a sample include the Folsom plankton splitter, and the stempel pipette, both of which were used in the present study. While Van Guelpen *et al.* (1982) suggested that the Folsom splitter was the most precise and accurate of the available sub-sampling devices, there are two assumptions that must be fulfilled when using this method. Firstly, each organism must have an equal probability of entering either chamber when making a split: this is ensured in part by adjusting the level of the splitter until equal volumes of

water are retained in each chamber. Statistical problems will arise with sequential splitting if this is not the case, with any errors in one split being built into all subsequent splits. Secondly, the movement of each organism must be independent of all the others: this is often invalidated when appendages become intertwined and clumps are formed. Regarding both assumptions, stirring the sample before the split is made helps to ensure a homogeneous distribution, such that clumps are broken up and an individual has an equal chance of entering either chamber. However, care must be taken to stir in a 'random' fashion so that centrifugal force does not bias the distribution of the sample in any way. While Van Guelpen *et al.* (1982) also found the stempel pipette to be a particularly fast and precise method of sub-sampling, they did warn that larger organisms may tend to get clogged in the open mouth, potentially causing problems with the undersampling of certain taxa.

3.4 Bioacoustical oceanography

3.4.1 Basic principles of sound energy

Sound is being used increasingly as a tool in zooplankton sampling programmes. This application has been termed "bioacoustical oceanography" (Greene *et al.*, 1998).

The properties of sound energy

Sound (acoustic) energy takes the form of a pressure wave, similar to shallow-water ocean waves, with alternating zones of compression and rarefaction. Sound waves can therefore be characterised by their amplitude (their height, which corresponds to their intensity, or loudness), their frequency (f, the number of waves that pass by in a given time) and their wavelength (λ, the distance between two successive crests or troughs). As speed is a function of distance and time, f and λ are related to speed (c) by:

$$c = f\lambda$$

Equation 3.3

Intensity (loudness) is proportional to the amplitude squared, and is measured in decibels (dB). The decibel scale is exponential, such that a 20 dB sound, for example, has a sound intensity factor of 10^2, while a 30 dB sound has an intensity factor of 10^3. Similarly, a –20 dB sound has an intensity factor of 10^{-2}, and so on. Frequency (pitch) is measured in hertz (Hz), which represents the number of waves, or cycles, per second. Frequencies above ~20 kHz cannot be heard by the human ear.

Sound energy loss

As sound waves are emitted uniformly in all directions from their point source, they spread out in ever-increasing circles. In water, the amplitude (acoustic intensity) of these waves decreases with increasing distance due to (1) spreading loss (proportional to the square of the distance travelled), (2) attenuation due to absorption (the conversion of acoustic energy into heat and chemical energy), and (3) scattering (reflection by suspended particles and air bubbles). Spreading loss and scattering are largely independent of frequency, while absorption is not. At higher frequencies (>10 kHz), the viscosity of the water is the main cause of absorption, while at medium (1-10 kHz) and low (<1 kHz) frequencies in seawater absorption is mainly due to the dissociation of both $MgSO_4$ ions and the $B(OH)_3$ complex (this process is also known as relaxation).

Sound energy in relation to different materials

The speed, *c*, of a sound wave through a given material is related to both the density and the axial modulus (elasticity) of that material (i.e. its ability to regain its shape following

compression, as well as its ability to resist compression). Solids have a greater axial modulus than liquids, and liquids greater than gases. Since the axial modulus and density of seawater are influenced by temperature (T), salinity (S) and pressure (related to depth, d), the speed of sound in seawater is also related to these variables. From 6 to 17 °C, the speed of sound in seawater (c, m s^{-1}) may be calculated from the equation;

$$c = 1410 + 4.21T - 0.037T^2 + 1.14S + 0.018d$$

Equation 3.4

The way in which sound behaves in a given material, its acoustic impedance (Z), is related to the density (ρ) and speed of sound (c) through that material by the equation;

$$Z = \rho c$$

Equation 3.5

Sound waves are reflected, or backscattered, more strongly at the interface between two materials which have very different values of Z. Reflectivity (R) is therefore given by;

$$R = \frac{Z_1 - Z_2}{Z_1 + Z_2} \times 100\,\%$$

Equation 3.6

where Z_1 and Z_2 represent the acoustic impedance of the materials on either side of the interface. Reflectivity is therefore a measure of the scattering strength, or target strength (TS), of an object that has been ensonified with acoustic energy. Put another way, the properties of any given material govern the intensity of the echo that it will produce.

The size of particles in relation to their acoustic detectability

Particles that are smaller than the wavelength of a given pulse of sound energy will cause relatively little of this energy to be backscattered (Wiebe *et al.*, 1990), or, as Francis *et al.* (1999a) wrote, "optimum scattering strengths (of smaller zooplankton) occur at higher frequencies, where the wavelength is comparable to, or smaller than, the dimensions of the structure". Wavelength is not only governed by frequency, but also by the medium through which the sound is travelling. For example, in surface (0 m) seawater at 12 °C and 33 psu, the speed of sound is 1492.8 m s^{-1} (see Equation 3.4). Under these conditions, the wavelengths of sound waves at various frequencies (from Equation 3.3), and therefore the acoustic detection threshold size, will be as follows: 38 kHz = 39.28 mm; 120 kHz = 12.44 mm; 150 kHz = 9.95 mm; 300 kHz = 4.98 mm; 600 kHz = 2.49 mm. At depth (e.g. 100 m), where temperature is lower (e.g. 7 °C) and salinity is higher (e.g. 34 psu), the speed of sound will be slower (1478.2 m s^{-1}) and the wavelengths will therefore be shorter: 38 kHz = 38.90 mm; 120 kHz = 12.32 mm; 150 kHz = 9.85 mm; 300 kHz = 4.93 mm; 600 kHz = 2.46 mm. However, as Wade & Heywood (2001) pointed out, even if the wavelength is longer than a given individual particle, "this wavelength can produce significant scattering from closely spaced groups of individuals".

3.4.2 The use of acoustics in oceanographic research

It has long been recognised that the ability to detect small particles in the ocean with sound energy might be of considerable use in oceanographic research (e.g. Greenlaw, 1979). While acoustical sampling does not allow precise identification of targets, its advantage lies in the generation of synoptic-scale information with real-time coverage.

Fisheries research

MacLennan & Holliday (1996) reviewed the use of acoustics in fisheries and plankton research. Sound energy was first used in an oceanographic capacity in the 1940s to detect the simple presence or absence of fish. By the 1960s, estimates of fish abundance were starting to become possible with the use of echo counters and echo integrators. In the 1970s, much of the research was focused on trying to understand the acoustic target strength of fish (e.g. Nakken & Olson, 1977) and the problem of 'echo integration', in which it was assumed that the amount of backscatter received from more than one fish target was the sum of the backscatter that would have been received from each individual in isolation. Following much argument, the validity of this 'linearity principle' was finally proven by the field experiments of Foote (1983). In the 1980s, attention was given to improving calibration techniques and to the development of dual-beam and split-beam echosounders that allowed the target strength of fish to be measured *in situ*. By the late 1980s, it had become apparent that statistical techniques needed to be developed in order to interpret the results of acoustic fish surveys accurately.

Plankton research

As Roe & Griffiths (1993) explained, "techniques for analysing zooplankton have lagged behind developments in fisheries acoustics, partly because of the technical difficulties involved and partly because of the rather generalised nature of the information obtained". One of the earliest accounts of the use of acoustics in plankton research was by Northcote (1964), who used a high-frequency echosounder to detect *Chaoborus* larvae. Other accounts around this time included those of Bary (1966), McNaught (1968, 1969) and Barraclough *et al.* (1969). McNaught *et al.* (1975) were the

first to employ a multi-frequency principle in order to estimate the biomass and size distribution of zooplankton populations from acoustic backscatter. These estimates were improved upon by Greenlaw (1977, 1979) and Johnson (1977), who began to develop models for the scattering strengths of different groups of zooplankton, and statistical procedures for the analysis of backscatter data. Acoustic research since the late 1970s has principally involved:

1. The concurrent use of standard zooplankton collection and enumeration techniques (e.g. nets, pumps) in order to validate acoustic data (e.g. Everson, 1982; Pieper & Holliday, 1984; Falk-Petersen & Kristensen, 1985; Costello *et al.*, 1989; Flagg & Smith, 1989; Heywood *et al.*, 1991; Madureira *et al.*, 1993; Buchholz *et al.*, 1995; de Robertis, 2001; Liljebladh & Thomasson, 2001; Wade & Heywood, 2001; Tarling *et al.*, 2002).
2. The continued development of multi-frequency techniques for the calculation of abundance and the differentiation of various scattering types (e.g. Greenlaw & Johnson, 1983; Pieper & Holliday, 1984; Kristensen & Dalen, 1986; Costello *et al.*, 1989; Holliday *et al.*, 1989; Everson *et al.*, 1993; Madureira *et al.*, 1993; Stanton *et al.*, 1994; Atkins *et al.*, 1998).
3. The development and use of target strength models based on theoretical, experimental and field data (e.g. Greene *et al.*, 1989; Stanton *et al.*, 1994; Martin *et al.*, 1996; Trathan *et al.*, 1995; Francis *et al.*, 1999a, b; Stanton & Chu, 2000; de Robertis, 2001).

The references included here represent only a percentage of the available literature, much of which is to be found in specialised engineering and acoustics journals. Two

marine journal volumes (ICES Journal of Marine Science, vol. 53, 1996, and Deep-Sea Research II, vol. 45, 1998) have actually been dedicated purely to bioacoustical contributions, and a comprehensive review of plankton acoustic studies has recently been provided by Foote & Stanton (2000).

The acoustic Doppler current profiler (ADCP)

The acoustic Doppler current profiler (ADCP) is a specialised type of echosounder, designed originally in the 1980s for use by physicists in the measurement of water movements (see review by Woodward & Appell, 1986). The ADCP works on the principle that particles in the water, which are assumed to be drifting passively with the prevailing current, may be detected from their backscatter. Furthermore, measurement of the Doppler shift* in the frequency of this backscatter will allow the relative movement of these particles, and hence the water current, to be quantified. By having four transducers pitched at different angles, the ADCP is able to determine movement in any three-dimensional plane. As Haury & Wiebe (1982) first pointed out, since these particles will be primarily zooplankton and micronekton in the open ocean, one should also be able to infer biological information using this instrument.

Flagg & Smith (1989) carried out the first field-test of a slightly modified 300 kHz narrowband† ADCP in a biological capacity. As they explained, the intensity of the backscattered signal, or the echo amplitude, is measured by the instrument's transducers

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* A change in sound wave frequency results when the source (i.e. the ensonified particle) and the observer (i.e. the ADCP instrument) are in relative motion. As the sound source approaches the observer the sound waves compress and the pitch rises, and vice versa. This change in pitch is known as the *Doppler effect*, named after Austrian physicist Christian Doppler.

† Narrowband acoustic instruments generate sound pulses over a smaller range of frequencies than broadband instruments.

as a voltage output. The Doppler processing hardware requires that this voltage is constant, to which end the echo amplitude is increased by the appropriate amount by an automatic gain control (AGC) amplifier. The degree of this amplification is therefore a measure of the strength of the backscatter, and hence the quantity and vertical distribution of what is most likely to be zooplankton. With broadband instruments, echo amplitude is related to a similar parameter, the Received Signal Strength Indicator (RSSI).

Since this pilot study, the ADCP has been increasingly used in a biological capacity. Roe & Griffiths (1993) presented data from a 150 kHz ADCP deployed in the NE Atlantic "in the hope that this will stimulate other users to make use of a potentially valuable source of data". Many studies have detected patterns of vertical migration, demonstrating that the ADCP is a useful tool in ecological studies of this behaviour (e.g. Pleuddemann & Pinkel, 1989; Roe & Griffiths, 1993; Zhou *et al.*, 1994; Cochrane & Sameoto, 1994; Buchholz *et al.*, 1995; Batchelder *et al.*, 1995; Roe *et al.*, 1996; Heywood, 1996; Tarling *et al.*, 1998, 2002; Pinot & Jansa, 2001; Liljebladh & Thomasson, 2001). However, we must also be aware of the limitations of the ADCP as a quantitative tool, as stated by Brierley *et al.* (1998): "The numerous uncertainties surrounding ADCP calibration and the current practical impossibility for users to monitor system performance should, however, preclude these instruments from being used as a matter of course to determine abundance estimates, a task that we believe should remain firmly within the domain of a well calibrated scientific echo sounder". Despite recent improvements in calibration, which are increasing the ability of the instrument to measure biomass, it is fair to say that this *caveat* still applies, and that uncertainties remain as to the accuracy of the ADCP relative to the traditional echosounder.

4

THE VERTICAL MIGRATIONS OF PREDATOR AND PREY IN A SCOTTISH FJORD: *CALANUS* (CRUSTACEA: COPEPODA) AND KRILL (CRUSTACEA: EUPHAUSIACEA) IN THE CLYDE SEA

4.1 Introduction

4.1.1 A history of research in the Clyde Sea Area

The "Clyde Sea Area" (Figure 4.1) has been defined as those bodies of seawater that lie "within a line drawn from the Mull of Kintyre to the Rinns of Galloway" (Mill, 1901). Boyd (1986) provided an interesting account of the socio-economic history of this region since the 'industrial revolution' of the mid-18th century. "A great industrial and sea-faring tradition" began with the invention of the steam engine in Glasgow in 1776, and the subsequent construction on the Clyde in 1812 of Bell's 'Comet', the world's first steamboat. While the words "Clyde-built" and "Clyde-navigation" became synonymous with "high quality workmanship and astuteness in business", the rapid growth of Glasgow and surrounding areas which continued until the 1970s meant that, for a time, the Clyde "became an open sewer", and the word "Clydeside" became synonymous with "dense acrid fogs which greatly affected the health of the people". Fortunately in latter years, our increasing awareness of, and pro-active approach to, environmental issues, has resulted in something of an 'environmental revolution', and the natural environment of the Clyde Sea Area appears to be recovering. Salmon (*Salmo salar*) and sea trout (*S. trutta*) have returned to the River Clyde, and, in 1982, a common dolphin (*Delphinus delphis*) was even seen in Princes Dock in the centre of Glasgow.

According to Chumley (1918), we can trace oceanography in Scotland back to "one Sunday in the month of May, 1883" when "Mr. (afterwards Sir) John Murray, of the 'Challenger' Expedition, was walking along the sea-shore...near Edinburgh". As the story goes, it was decided to build a floating laboratory, the "Ark", in a disused quarry near Edinburgh. With support for the idea from his scientific colleagues ("It seems to me a good one, and I'll build your floating laboratory for you, if it will not cost over £1000"), the Scottish Marine Station (SMS) was born on March 15th 1884. It is also

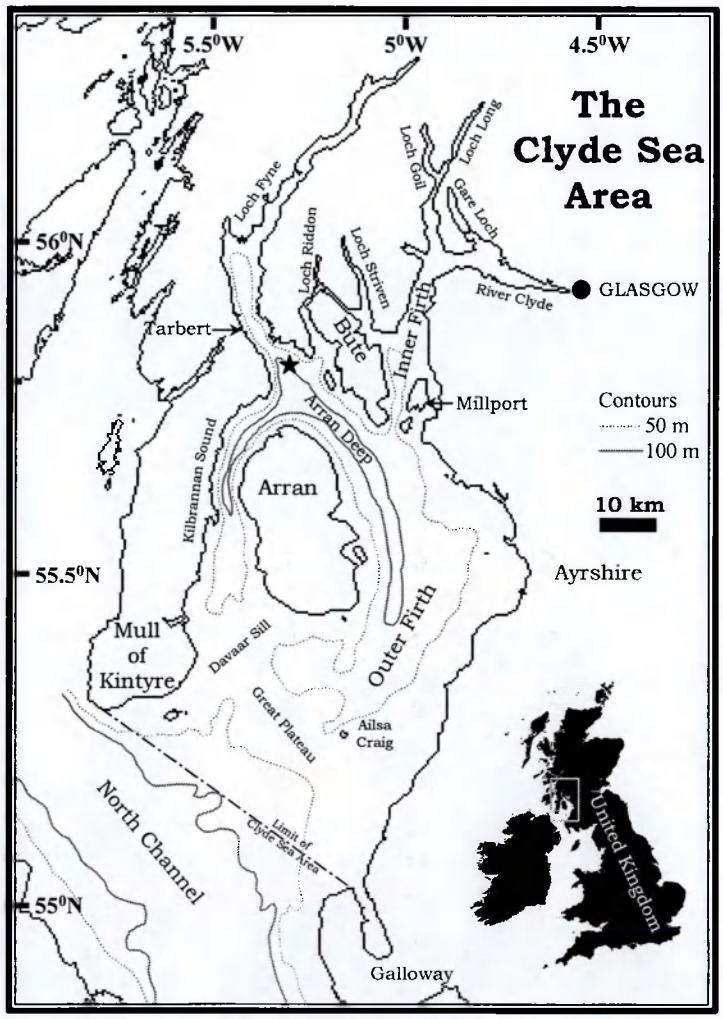

Figure 4.1 The Clyde Sea Area and North Channel. ★ shows the location of sampling in Inchmarnock Water during the present study. Bathymetric contours taken from Edwards *et al.* (1986).

good to know that the SMS was an 'equal'-opportunity employer, since the "Ark" was fitted with "a commodious laboratory for ordinary use" and "a smaller one…for any lady-workers who should desire to use it". In June 1885, the SMS extended summer operations to Millport in the Clyde Sea Area, with the "Ark" and Murray's steam yacht "Medusa" being towed "thither" via the Forth and Clyde Canal. With the additional establishment earlier that year of a temporary laboratory at Tarbert on Loch Fyne by the Fishery Board for Scotland, oceanographic studies in the Clyde began in earnest. The "Medusa" was used almost continuously until 1892 for studies of the physical and biological environment, although only the physical data were ever published, while the

Fishery Board conducted studies of the herring fishery for which Loch Fyne was renowned. The Scottish Marine Biological Association (SMBA) was established in 1894, when the "Ark" was taken over by the Millport Marine Biological Station Committee, and continues to the present day. Much of what we know about the environment of the Clyde Sea can be attributed to the extensive work of the SMBA.

4.1.2 Clyde Sea hydrography

H. R. Mill has been credited with the first hydrographic studies in the Clyde Sea Area (e.g. Mill, 1892, 1901). A number of further studies have taken place since this time (e.g. Chumley, 1918; Barnes & Goodley, 1961; Edwards *et al.*, 1986; Simpson & Rippeth, 1993; Jones *et al.*, 1995; Muller *et al.*, 1995a, b; Rippeth *et al.*, 1995; Rippeth & Simpson, 1996; Rippeth & Jones, 1997; Matthews *et al.*, 1999). The following descriptions are taken from Edwards *et al.* (1986) and Simpson & Rippeth (1993).

The Clyde Sea Area is the largest fjord system in Scotland, featuring reduced surface salinities, summer stratification, and winter renewal of deep-water masses. The stratified Outer Firth water is separated from the well-mixed North Channel water by the Great Plateau, across which periodic inflows occur. The deep-water trenches of Kilbrannan Sound (165 m) and the Arran Deep (160 m) are bordered at their southern ends by the Davaar Sill (40 m) and the Great Plateau (50 m), respectively. These two trenches meet at Inchmarnock Water (195 m) to the north of Arran, and continue north-westwards to the Otter Point sill (30 m) in Loch Fyne. The Bute sills (25-45 m) separate Lochs Riddon, Striven, Goil, Long, and Gare Loch from the rest of the Clyde Sea Area. It is principally the bathymetry that controls the hydrography at any given depth: the surface waters are freely connected (although often isolated from the North Channel by a strong front in the summer months) and controlled by tide, wind and freshwater

outflows, while the deeper waters (below sill depth) are more isolated and controlled by oscillatory internal movements, weak diffusion and downward density flows. The nutrient-rich freshwater inflows, which may be significant (from 140 to 1900 × 10^6 m^3 $month^{-1}$), arise from the River Clyde, Loch Lomond, the lochs and catchments north of the Bute sills, the Ayrshire rivers and the Outer Firth catchments (i.e. Arran and the Mull of Kintyre).

The deep saline waters (>50 m) of Kilbrannan Sound and the Arran Deep exchange with the surface waters by vertical diffusion and convection. From October to March, the deep waters are frequently renewed by Plateau water, but become isolated below a pycnocline (at ~40 m) from May to September. Overall, the hydrographic system is driven by the competition between freshwater and thermal buoyancy inputs, and mechanical stirring caused by wind-stress, tidal forcing (which is relatively weak in the Clyde Sea) and convection. The slow vertical diffusion of surface waters to the depths during the winter months (driven by wind and, to a lesser extent, tide) is periodically enhanced by deep-reaching convective inflows of dense water originating from the North Channel. Annual surface temperatures range from ~6 °C in March to ~15 °C in August, while deep-water temperatures range from ~6 °C in March to ~12 °C in November. Salinities in the freshwater-influenced surface waters range from ~31.8 to ~33.2 psu, while deep-water salinities range from ~33 to ~34 psu. The water in the Arran Deep is completely renewed roughly every month (except when stratified), implying a mean flow rate of ~10^4 m^3 s^{-1}, with a mean northward speed of 3 cm s^{-1} and an uplift rate (at 50 m) of ~1 m d^{-1}. The surface waters (<50 m) of the Outer Firth exchange at ~1.6 × 10^4 m^3 s^{-1} with the North Channel, resulting in a residence time of ~2 months.

4.1.3 Primary production and phytoplankton in the Clyde Sea

An early account of phytoplankton in the Clyde Sea Area was given by Murray & Blackman (1901), based almost entirely on a Fishery Board for Scotland report written by G. Murray in 1857. A number of further studies have taken place since this time (e.g. Marshall, 1924; Marshall & Orr, 1927, 1930; Hinton, 1974; Marshall & Boney, 1974; Hannah & Boney, 1983; Tett *et al.*, 1986; Jones *et al.*, 1995; Rippeth & Jones, 1997). As Hannah & Boney (1983) discussed, most such studies have considered the phytoplankton as a homogeneous unit, and therefore overemphasised the importance of the diatoms and dinoflagellates ("netplankton") to primary production. Since their study from the Clyde Sea also considered the "nanophytoplankton" (i.e. those photosynthetic organisms <20 μm and therefore too small to be captured by net sampling), the description given below is based for the most part on their findings. While these samples were actually collected from near Millport, no such detailed studies appear to have been undertaken in Inchmarnock Water and it is assumed that conditions at both sites will be similar.

Hannah & Boney (1983) found that the levels of chlorophyll *a* were a good indicator of carbon-fixation (= primary production). Primary production was highest in the top 1 m of the water column, and decreased rapidly towards ~10 m depth. Tables VII and VIII in Hannah & Boney (1983) show the species composition of the nanophytoplankton (<20 μm) and netplankton (>20 μm), respectively. During the winter months (November to March), phytoplankton biomass and chlorophyll *a* levels were low, and primary production ranged from 0 to 5 mg C m^{-3} h^{-1}. Nanophytoplankton <5 μm (e.g. green flagellates, cryptomonads and *Pyramimonas* spp.) were the main contributors to both biomass and chlorophyll *a*, while levels of surface nutrients (silicate, nitrate and phosphate), fuelled mainly by North Channel inflows (Jones *et al.*,

1995), were high at this time. A spring diatom increase in March/April appeared to be triggered by ~14 d of calm weather and high levels of sunshine, while persistent high winds through late winter/early spring were suggested to delay this increase and/or affect the magnitude of diatom growth. This 'spring bloom' was dominated by *Skeletonema costatum* and *Thalassiosira nordenskioldii*, with other diatoms present including *Nitzschia seriata*, *Chaetoceros* spp. and *Phaeocystis pouchetii*. Levels of chlorophyll *a* at this time peaked at ~10 mg m^{-3}, and primary production ranged from 30 to 40 mg C m^{-3} h^{-1}. Following a rapid decline in nutrient levels, the post-bloom period (late May) featured a return to nanophytoplankton-dominated (3-12 μm) conditions, with groups present including prasinophytes, prymnesiophytes, cryptophytes and chrysophytes (especially *Apedinella spinifera*). These groups contributed up to 87 % of the chlorophyll *a* levels at this time. The summer period (June to early August) was punctuated by peaks in chlorophyll *a* levels, driven by fluvial nutrient input and vertical mixing of nutrients from deep water. There was a diatom increase in June, consisting of *Chaetoceros* spp., *S. costatum* and *Rhizosolenia deliculata*, after which (mid-July) dinoflagellates (e.g. *Dinophysis acuta*, *Peridinium* spp., *Ceratium* spp.), small non-thecate species and green flagellates (<5 μm) took over. A further diatom increase at the end of July consisted of *Ceratulina pelagica*, *Chaetoceros* spp., *Thalassiosira* spp. and *R. deliculata*, as well as green flagellates <5 μm. Primary production during these summer blooms was higher (up to 65 mg C m^{-3} h^{-1}) than during the spring bloom. In early September, diatoms such as *Leptocylindricus danicus* and *Chaetoceros* spp., non-thecate dinoflagellates (<15 μm) and green flagellates (<5 μm) were present. An autumn (late-September) 'pulse' of diatoms, including *Nitzschia* spp., *S. costatum*, *T. nordenskioldii*, *R. deliculata* and *Eucampia zodiacus*, was evidenced by an increase in the levels of chlorophyll *a*. Primary production during this pulse was on the order of 20

mg C m^{-3} h^{-1}. From September onwards, the chlorophyll *a* levels remained low, at <0.5 mg m^{-3}, while the levels of nutrients at the surface began to increase as the nutrient-rich deep-water masses were displaced during winter mixing.

4.1.4 Clyde Sea zooplankton

Past studies

Adams (1986) reviewed the zooplankton studies that have been carried out in the Clyde Sea Area, and identified six main groups of investigators;

1. The Fishery Board for Scotland, 1885-1889 (e.g. Brook, 1886; Calderwood, 1886; Williamson, 1899; Scott, 1907, 1909).
2. Sir John Murray and colleagues, 1885-1892 (e.g. Murray, 1888).
3. The Scottish Marine Biological Association, 1894-1982 (e.g. Marshall, 1925, 1949; Macdonald, 1927, 1928; Marshall *et al.*, 1939; Rees, 1941; Bainbridge, 1958; Mauchline, 1966; Edwards, 1978; Grigg & Bardwell, 1982).
4. Sir William Herdman and colleagues, 1907 (e.g. Herdman & Riddell, 1910).
5. Sir Alister Hardy and colleagues, 1941-1943 (see SMBA annual reports 1941/42 to 1943/44).
6. The Marine Laboratory, Aberdeen, 1970-1974 (e.g. Johnston *et al.*, 1974).

A more recent study has been conducted as part of the EU MAST project, Impact of a Climatic Gradient on the Physiological Ecology of a Pelagic Crustacean (PEP) (Buchholz *et al.*, 1998).

The mesozooplankton community

The Clyde Sea has a rich and varied mesozooplankton community that is dominated by pelagic crustacea. Of these, the copepods are the most important group, both in terms of numerical abundance, and as food for commercially important fish such as herring (*Clupea harengus*), mackerel (*Scomber scombrus*), Norway pout (*Trisopterus esmarkii*) and larval plaice (*Pleuronectes platessa*). Of the copepods, the omnivorous species *Calanus finmarchicus* and *C. helgolandicus* appear to be the most important within the ecosystem, followed by the primarily herbivorous species *Pseudocalanus elongatus*, *Acartia clausi* and *Oithona similis*, and the carnivorous species *Euchaeta norvegica*, *Centropages hamatus* and *Temora longicornis*. Other species include *Microcalanus pygmaeus* and *Paracalanus parvus*. Two krill species, *Meganyctiphanes norvegica* and *Thysanoessa raschii*, are common in the Arran Deep, Kilbrannan Sound and Loch Fyne, where they perform strong vertical migrations (e.g. Macdonald, 1928; Mauchline, 1960). *Thysanoessa inermis* is rare, and *Nyctiphanes couchii* may appear if advected across the Great Plateau in autumn. *M. norvegica* is omnivorous, filter-feeding on diatoms, dinoflagellates and resuspended benthic detritus, and raptorially feeding on copepods such as *Calanus* and *E. norvegica*, other krill and chaetognaths (e.g. Mauchline, 1966, 1980; Adams, 1986; Båmstedt & Karlson, 1998; Lass *et al.*, 2001). *T. raschii* has a similar diet (except for other krill), but feeds more on plant and detrital material (e.g. Mauchline, 1966, 1980; Sameoto, 1980). In turn, these krill species provide food for planktivorous fish such as herring, mackerel, and hake (*Merluccius merluccius*).

The cladoceran species *Evadne nordmanni*, *Podon leuckarti*, *P. intermedius* and *P. polyphemoides* are often numerous in the surface waters, where they feed on tintinnids, peridinians and other microplankton. Cirripede (Barnes, 1956) and decapod larvae may

prevail at certain times of the year, as may mysids (Mauchline, 1971). The ctenophores *Beroë cucumis*, *Pleurobrachia pileus* and *Bolinopsis infundibulum* may be so abundant in some years that large portions of the *Calanus* stock are decimated. Other zooplankton species present include the appendicularians (larvaceans) *Fritillaria borealis* and *Oikopleura dioica*, the chaetognaths *Sagitta elegans* and *S. setosa*, and a variety of meroplanktonic benthic invertebrate larvae. The decapod shrimp *Pasiphaea multidentata* may also occur, and was found on a few occasions in the present study.

Calanus finmarchicus* and *C. helgolandicus

Calanus finmarchicus and *C. helgolandicus* are probably the most important members of the mesozooplankton community in the Clyde Sea in terms of acting as a crucial link between primary production and higher predators. Indeed, due to its abundance, *C. finmarchicus* is widely regarded as the most important mesozooplankton species in the whole of the North Atlantic. Given the focus of the present study, it is therefore fitting to provide a more detailed introduction to *Calanus* at this point. The often-cited monograph, "The Biology of a Marine Copepod" (Marshall & Orr, 1955) remains as the most seminal reference to date on both *C. finmarchicus* (Gunnerus) and *C. helgolandicus* (Claus), while studies such as those of Williams & Conway (1982, 1984) and Bottrell & Robins (1984) on *C. helgolandicus* from the Celtic Sea have gone some way towards redressing the balance of research that has been overwhelmingly in favour of *C. finmarchicus*. Marshall & Orr (1955) presented a fascinating account of the systematics of both species, including the initial discovery of *C. finmarchicus* in 1767 (Gunnerus, 1770) (Figure 4.2), the eventual resolution of the various synonymies that ensued for over 100 years (Giesbrecht, 1892), the derivation of the name "Calanus" (Leach, 1819) and the recognition (Claus, 1881) and naming (Sars, 1903) of the subtly

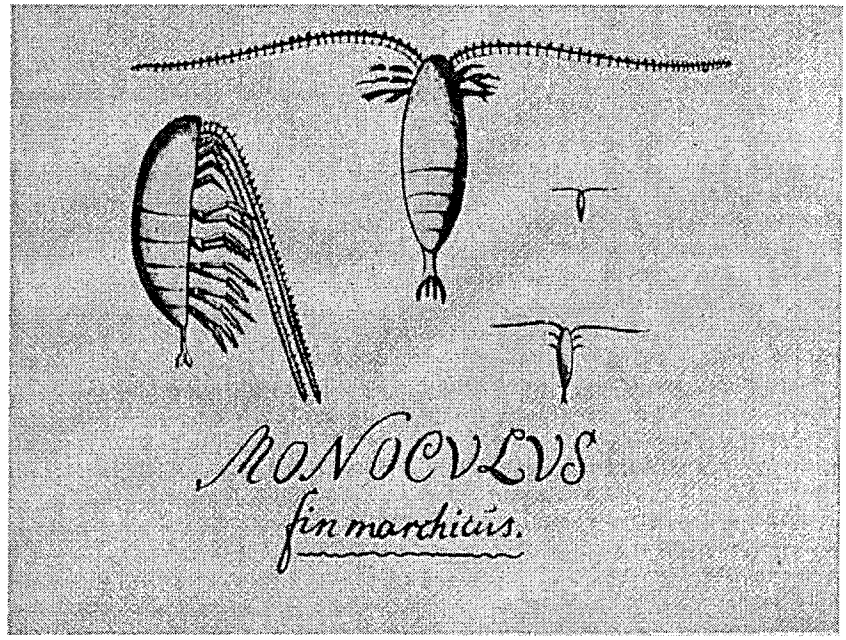

Figure 4.2 The first sketches of *Calanus finmarchicus* by Gunnerus (1770). This species was originally called *Monoculus finmarchicus*, until the general confusion and synonymies surrounding this animal were resolved by Giesbrecht (1892). The smallest drawing shows the actual size. Reproduced from Fig. 1 in Marshall & Orr, 1955.

different *C. helgolandicus* as a separate species (although this wasn't widely recognised until Rees, 1949). Given the fact that *C. finmarchicus* and *C. helgolandicus* are not readily distinguishable from each other in the field (see Mauchline, 1956, for a description of the diagnostic features), and that both are known to co-exist in the Clyde Sea Area (Marshall & Orr, 1955), the samples collected during the present study were likely to have contained a mixture of both species (although no *C. helgolandicus* were identified in subsamples that were checked for specific identification: J.B.L. Matthews, pers. comm.). When discussing these samples, the generic term *Calanus* will therefore be used in recognition of this fact.

The distributions of both *C. finmarchicus* and *C. helgolandicus* described by Marshall & Orr (1955) are now generally thought to be inaccurate, due to confusion with the similar species *Calanus glacialis* in some regions in the northern hemisphere,

and with *C. australis* and *C. pacificus* in all regions the southern hemisphere (J.B.L. Matthews, pers. comm.). A more accurate assessment of the distributions of *C. finmarchicus*, *C. helgolandicus,* and *C. glacialis* in the North Atlantic was given by Matthews (1967). In general, *C. finmarchicus* prefers colder temperatures, and *C. helgolandicus* warmer temperatures (e.g. Williams, 1985).

Studies into the vertical distribution of *C. finmarchicus* and/or *C. helgolandicus* are numerous and date back maybe a century or more. As Williams & Conway (1982) pointed out, most of the early investigations, with the exception of those of Russell (e.g. 1927) from the English Channel, involved *C. finmarchicus* (e.g. Gardiner, 1933; Nicholls, 1933; Marshall *et al.*, 1934). The one conclusion that seems common is that the VM behaviour of *Calanus* is highly variable, over both diel and seasonal time scales, and often both between and among developmental stages. Given its extraordinary behavioural plasticity, it is hard to pinpoint any generally accepted trends (e.g. Pearre, 2000), and even harder to predict how *Calanus* might behave under a given set of environmental conditions. Suffice it to say, *Calanus* is certainly capable of migrating over several hundreds of metres. Upward swimming speeds for *C. finmarchicus*, as measured in the famous "plankton wheel" (Hardy & Bainbridge, 1954), are 4 mm s^{-1} for prolonged (1 h) periods and 18 mm s^{-1} for short (2 min) periods, while downward swimming speeds are 13 mm s^{-1} (1 h) and 30 mm s^{-1} (2 min). Minkina (1981) found the swimming speed of *C. helgolandicus* to be marginally higher (5 to 40 mm s^{-1}). No doubt the faster downward swimming speeds are aided by passive sinking, which, for an animal with a density in the region of 1.043-1.047 g cm^{-3} (*C. finmarchicus* adult females: Gross & Raymont, 1942), has been found to range from 0.8 to 8 mm s^{-1} (Apstein, 1910; Gardiner, 1933; Gross & Raymont, 1942; Landry & Fagerness, 1988).

The food and feeding of *Calanus* have been investigated by a variety of authors (e.g.

Marshall, 1924; Butler *et al.*, 1969; Joint & Williams, 1985; Simard *et al.*, 1985; Tande & Bamstedt, 1985; Irigoien *et al.*, 1998; Pasternak *et al.*, 2001; Jonasdottir *et al.*, 2002). It has been clearly shown that *Calanus* is omnivorous, feeding on whichever microplankton are the most readily available at the time. Gauld (1951) showed that *Calanus* is capable of filtering organisms as small as 6 μm in diameter (*Chlamydomonas* sp.). For copepods in general, Jorgensen (1966) suggested that cells <30-50 μm across will be filtered, while larger cells will be caught raptorially. However, the upper size limit is less well known (Marshall, 1973). Marshall (1924) examined over 3000 individuals from the Clyde Sea during 1923 to investigate the seasonal changes in feeding and food items. During the winter, feeding was reduced to the nighttime period, when food items included diatoms such as *Coscinodiscus* sp. and *Biddulphia* sp. (when available) and the radiolarian *Acanthonia mulleri*. The proportion feeding by day increased with the onset of the spring bloom, with the main food eaten being the dominant diatoms *Skeletonema* sp. and *Thalassiosira* sp.. As summer progressed, equal proportions of the population fed both day and night. Food items during this time included diatoms such as *Navicula* spp., *Rhizosolenia* spp., and *Chaetoceros* spp., dinoflagellates such as *Peridinium* spp. and *Dinophysis* spp. and the coccolithophore *Pontosphaera huxleyi*. Moving into the autumn diatom pulse, the dominant food items were again *Skeletonema* and *Thalassiosira*, shifting more to *Coscinodiscus* and *Biddulphia* as the winter approached. Silicoflagellates such as *Distephanus* sp. were also eaten at this time. Crustacean remains were found at all times in the guts, with more being eaten during the winter and during the summer maximum. These were unidentifiable to genus or species level for the most part, although other copepods were occasionally identified. Other identifiable organisms in the guts included molluscan larvae and tintinnids.

4.2 Materials and methods

4.2.1 Sampling site and schedule

Sampling was carried out in Inchmarnock Water at the northern end of the Arran Deep in the Clyde Sea Area, western Scotland (55.81 °N, 5.26 °W) (Figure 4.1). Water depth at this location was ~160 m. This site was visited approximately every two months from June 1999 to June 2000 as part of a multi-disciplinary study to investigate bio-physical interactions within a fjordic environment (Figure 4.3).

Net tows and other deployments were made from the R/V "Calanus", a 25 m research vessel operated and maintained by the Scottish Association for Marine Science (SAMS) at Dunstaffnage Marine Laboratory (DML). A U-shaped mooring supporting a variety of instruments was deployed at the same site throughout the study period. Table 4.1 shows the dates of the ship visits, and the periods during which the moored instruments were deployed. Due to the site's proximity to shore, each of the seven ship

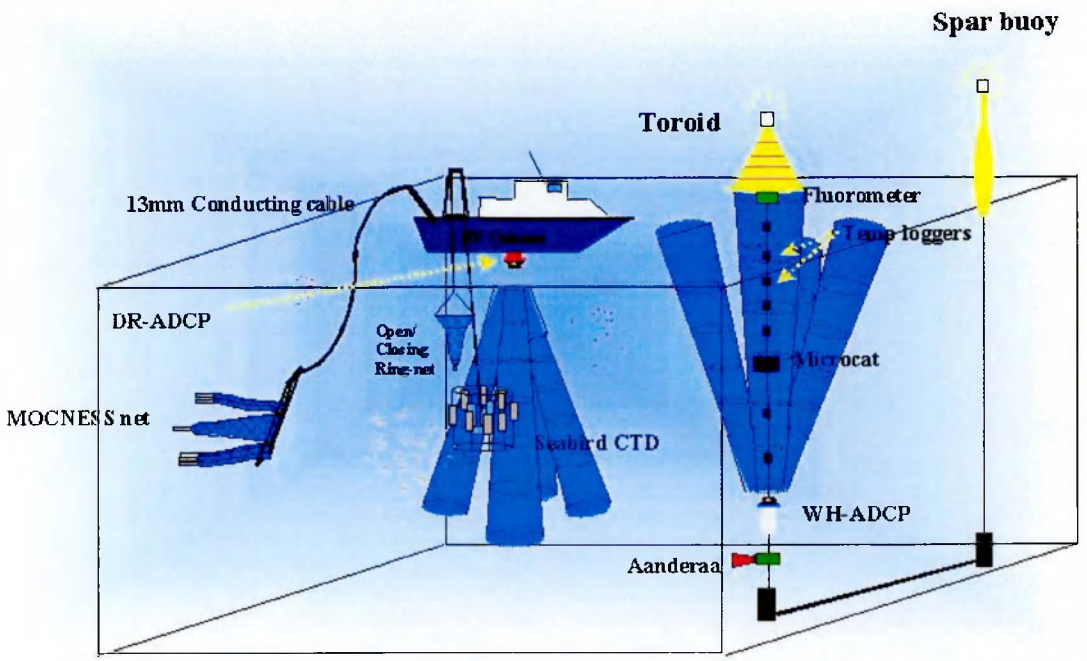

Figure 4.3 Diagram showing the main components of the 1999-2000 bio-physical sampling programme in Inchmarnock Water, of which the present study was a part.

Ship visits	Mooring deployments
Jun'99 = June 28 – July 2, 1999	June 24 – August 13, 1999
Aug'99 = August 9 – 13, 1999	August 16 – October 4, 1999
Oct'99 = October 4 – 8, 1999	October 8 – December 6, 1999
Dec'99 = December 6 – 9, 1999	December 9 – January 17, 2000
Jan'00 = January 17 – 20, 2000	January 19 – March 1, 2000
Mar'00 = March 1 – 7, 2000	March 8 – May 2, 2000
May'00 = May 8 – 12, 2000	May 3 – July 4, 2000

Table 4.1 Sampling dates during the 1999-2000 bio-physical sampling programme in Inchmarnock Water, of which the present study was a part.

visits was undertaken as a series of 6-8 h trips from the nearby harbour at Tarbert, allowing the crew time to rest in between trips. While bad weather meant that ship-based sampling in March 2000 had to be undertaken further north in the shelter of Loch Fyne (55.89 °N, 5.38 °W), all other ship visits were undertaken as near to the moored array as possible.

4.2.2 CTD and related measurements

From June to August 1999, the conductivity-temperature-depth (CTD) apparatus used was a Neil Brown "Smart" CTD, while from October 1999 onwards a Sea-Bird (SBE 9/11+) instrument package, mounted with a 12-position rosette, was used. This rosette was typically equipped with several 5-litre Ocean Test Equipment (OTE) bottles. With the Neil Brown, no other instrumentation was deployed in addition to the standard pressure and temperature sensors, conductivity cell and pump. With the Sea-Bird, additional instruments included a dissolved oxygen sensor, a transmissometer and a fluorometer. The temperature, conductivity, and oxygen sensors were connected to an SBE pump, which ensured that water passed the sensors at a known rate, independent of winch speed.

During deployment, both types of CTD package were lowered at 0.5 m s^{-1} to a maximum depth of 140 m, and hauled to the surface at 1 m s^{-1} using the starboard hydrographic winch. Water bottles were fired during the upcast. With the Sea-Bird package, water samples were taken from the near-bottom OTE bottle for calibration with a Guildline salinometer back at DML, while the temperature sensor was checked against an SIS reversing thermometer fired at a given depth. Data were acquired from the Neil Brown using Neil Brown CTDACQ software, and from the Sea-Bird using SeaSave software, which was stored on removable disk and on the onboard computer hard disk and processed back at DML. Data were processed for each instrument package using post-processing software supplied by the respective manufacturers. Vertical-processed profiles were derived from the downcasts.

4.2.3 Light measurements

A Li-Cor LI-200SA pyranometer sensor was secured to the roof of a building in Tarbert (~10 km from Inchmarnock Water) to measure direct and diffuse solar radiation (Watts m^{-2}) at 1 min intervals from June 1999 to June 2000. From this rooftop position there were no obstructions that could have affected the readings. Data were relayed to a laptop computer inside the building via a Li-Cor LI-1400 datalogger, and checked and downloaded during every sampling visit.

4.2.4 Chlorophyll *a* measurements

Levels of chlorophyll *a* were measured via three different methods. Firstly, a Chelsea-MkIII Aquatracka fluorometer mounted on the underside of the Toroid mooring buoy provided continuous measurements of chlorophyll *a* fluorescence at the sea surface (1.5 m) from June 1999 to June 2000. During each ship visit, the fluorometer was removed,

replaced with a second identical instrument, and returned to DML for downloading and routine maintenance before redeployment on the next mission. Secondly, during each ship visit, a sample of seawater was collected from 2 m depth using a 5 litre NIO water bottle. This was deployed in the open position by hand from the side of the ship, and closed with a messenger weight sent down the rope line. Replicate 200 ml aliquots of the water sample were filtered through a 24 mm GF/F filter using a low-suction pump. The filters were placed in a cryotube, stored in the freezer at –20 °C, and returned to DML where total chlorophyll *a* content was measured using High Performance Liquid Chromatography (HPLC). Thirdly, a time-series (1997-2001) of ocean-colour data, derived from the Sea-viewing Wide Field-of-view Sensor (SeaWiFS) ocean-colour satellite, was kindly made available to this study by P. Miller of the Remote Sensing Group at Plymouth Marine Laboratory. The data provided were 7 d averages taken from a 4.4 km pixel in the vicinity of the mooring in Inchmarnock Water (centred around 55.80 °N, 5.25 °W), converted to chlorophyll *a* biomass (mg m^{-3}) using the "bright pixel" atmospheric correction algorithm developed by Plymouth Marine Laboratory (Moore *et al.*, 1999), and the NASA "OC2V2" chlorophyll algorithm (O'Reilly *et al.*, 1998).

4.2.5 WP-2 net tows

A 1 m-diameter, 500 μm-mesh WP-2 net (Unesco, 1968) was deployed to collect *Calanus finmarchicus* and *C. helgolandicus* for biometric measurements at various times during their DVM cycle (Figure 4.4). Hereafter, these species will be referred to collectively as *Calanus* (see section 4.1.4). The net, which was equipped with a filtering cod end, could be opened and closed at discrete depths by means of an Ocean Test Equipment (OTE) double-trip release mechanism. Tows were undertaken primarily

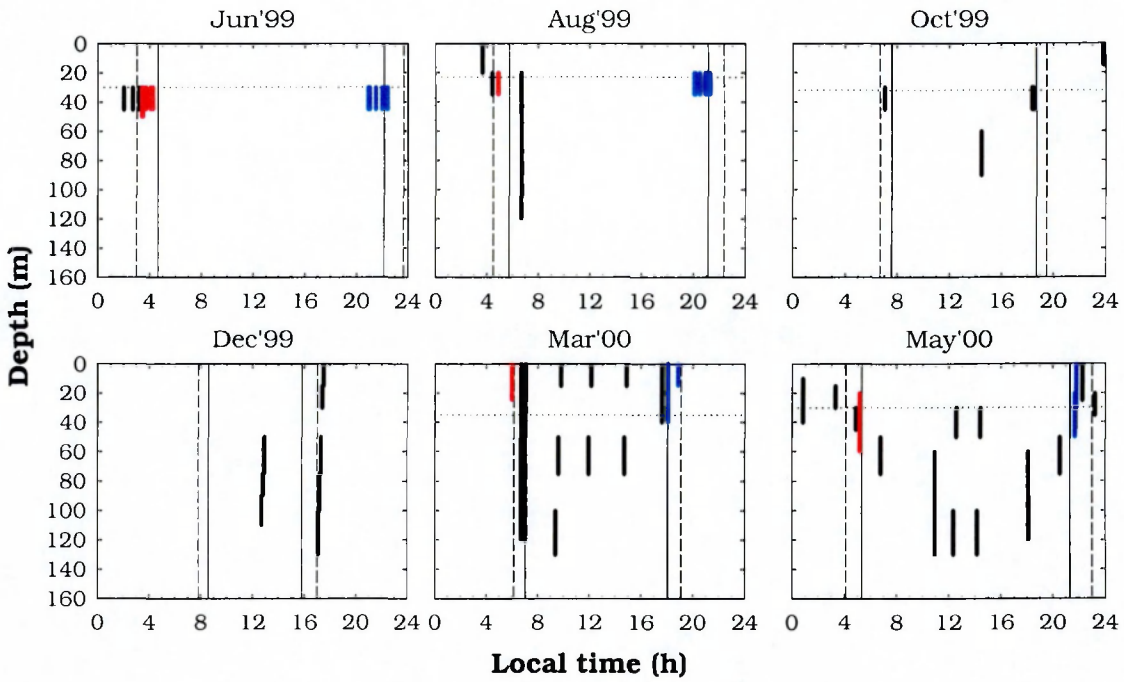

Figure 4.4 The time and depth of WP-2 net tows made in Inchmarnock Water for the collection of *Calanus*. The red and blue lines represent 'dawn' and 'dusk' net tows, respectively, and the thick black lines represent tows made at other times. The broken and solid vertical lines show the timings of first/last light and sunrise/sunset, respectively. The dotted horizontal line shows the deepest point of the pycnocline (when present).

around dawn and dusk, since this is when individuals were assumed to be performing their daily movements to and from the surface waters. Collections were also made at other times of day and night depending on the availability of time in the sampling schedule. The net was towed vertically (at approximately 0.6 m s^{-1}) from a stationary ship via the starboard hydrographic winch. At dawn and dusk, the net was towed open through the 15 m of water directly beneath the pycnocline (when present). The net was sent to its maximum depth in a bundled up, closed position, opened by sending a messenger weight down the wire, towed to the shallowest desired depth, and then closed with a second messenger. In the absence of any pycnocline during the winter months, and at times of day and night other than dawn and dusk, tows were made at depths approximate to the estimated depth of the migrating *Calanus* population.

Sample processing

Once the net was aboard, the contents of the cod-end were poured gently into a white plastic 2-litre bucket containing a small volume of carbonated, filtered seawater. This water was pre-prepared using a commercially available SodaStream™ carbonated-drinks maker. The dissolved carbon dioxide acted to anaesthetise the animals, making them easier to identify and sort, and minimising the stress of capture and handling. The bucket was taken into the onboard laboratory for immediate sorting and storage. The contents of the bucket were poured gently through a 5 cm-diameter, 500 μm mesh sieve, which was placed in a petri dish containing just enough filtered seawater to cover the bottom of the mesh. Under a binocular microscope, individual *Calanus* were sorted by developmental stage into CVI (adult) females, CV, and CIV, measured (prosome length) using an eyepiece graticule precise to ±0.04 mm, and picked out into pre-weighed Elemental Microanalysis Ltd. tin capsules (8 mm × 5 mm) with fine forceps. In March and May 2000, a visual estimate was also made of the gut fullness of individuals, as gut contents were visible through the semi-transparent body wall (this estimate was based on the relative length of the gut contents, and expressed on a scale of 0 to 100 by 5 % increments). For consistency, these estimates were carried out by myself throughout. Between one and six individuals of each stage were placed into any given capsule. The capsules, held in labelled 96-well microtitre plates, were stored in the freezer at –20 °C.

Back at DML, the frozen samples were oven-dried at 60 °C for ~48 h. The open tin capsules were placed in the oven in their microtitre plates with the lids removed. A layer of aluminium foil was laid loosely over the top of the samples to prevent the possibility of contamination from residues on the inside of the oven. After drying, the tin capsules were crunched into a sealed ball using forceps, and placed into labelled 2 ml Eppendorf

vials ready for elemental analysis. The dried samples in March and May 2000 were also weighed prior to elemental analysis on either a Mettler H20 or a Sartorius electrobalance, both of which were precise to ±10 μg. At the University of Wales, Swansea (UWS), the samples were combusted in a Europa Scientific Roboprep Biological Sample Converter (precise to ±0.01 μg) to yield their total carbon and nitrogen weights.

4.2.6 MOCNESS-1 net tows

A MOCNESS-1 net (Wiebe *et al.*, 1976b, 1985) was deployed to investigate the composition, concentration and vertical distribution of the mesozooplankton community (>300 μm), and to collect krill for biometric measurements at various times during their DVM cycle. The net was towed obliquely from a moving ship (speed through water ~2.5 knots) via the aft A-frame and an 11 mm conducting-cable winch. Tows were made at various times of the day and night, with up to nine remotely-controlled nets employed to sample discrete 15-30 m layers of the ~160 m-deep water column. Table 4.2 shows the four sampling protocols variously employed. Typically, the net system would be sent down to 130 m at a vertical speed of 0.5 m s^{-1} with net 1 open, and hauled-in between 0.3 and 0.5 m s^{-1}, stepping through nets 2 to 9 at the designated depth intervals using the onboard computer. Problems with the underwater electronics package in August 1999, however, meant that depth-discrete tows could not be made at this time. Therefore, depth-integrated mesozooplankton samples were obtained at four time-periods between 11:00 and 03:30 h via double-oblique tows, the net system being rigged so that two nets (a 300 μm-mesh and a 2 mm-mesh) were left permanently open, each with half the usual aperture (i.e. 0.5 m^2). For each time-period, three separate double-oblique tows were made in quick succession, paying out wire to 50, 100 and

	Protocol 1		Protocol 2		Protocol 3		Protocol 4	
Net	Mesh (mm)	Depth (m)	Mesh (mm)	Depth (m)	Mesh (mm)	Depth (m)	Mesh (mm)	Depth (m)
1	0.3	0-130	0.3	0-130	0.3	0-130	0.3	0-130
2	2	130-100	2	130-100	0.3	130-100	0.3	130-100
3	2	100-75	2	100-75	0.3	100-75	0.3	100-75
4	2	75-50	2	75-50	0.3	75-50	0.3	75-50
5	-	-	0.3	50-30	0.3	50-30	0.3	50-30
6	-	-	0.3	30-50	0.3	30-15	0.3	30-50
7	2	50-30	2	50-40	0.3	15-0	0.3	50-30
8	2	30-15	2	40-30	-	-	0.3	30-15
9	2	15-0	2	30-0	-	-	0.3	15-0

Table 4.2 Deployment protocols of the MOCNESS-1 net system in Inchmarnock Water between June 1999 and May 2000: the mesh size and tow depth of each of the 9 nets for 4 different sampling protocols.

then 150 m. Subsequent calibration hauls using a Vemco Minilog time/depth recorder (TDR) attached to the net frame showed that these tows would have fished at approximately 20, 40 and 60 m depth, respectively. In addition, due to this temporary technical hitch, two sets of depth-discrete tows were made using the WP-2 net described above in order to investigate the mesozooplankton community further (although krill would have been undersampled with this net due to avoidance behaviour). Depth-discrete tows were made in 20 to 30 m intervals between 130 m and the surface around 11:00 and 21:00 h.

Sample processing

Once the MOCNESS was aboard, the contents of the cod-ends were poured gently into labelled 10-litre plastic buckets and taken into the onboard laboratory. The contents of each bucket in turn were poured through a 20 cm-diameter, 500 μm-mesh sieve, and all of the larger krill specimens (>~10 mm) removed to a laminated sheet of graph paper (mm squares) with forceps. The graph squares were used to measure the body length

(middle of the eye to the end of the telson) of each specimen to the nearest mm. Specimens of *Meganyctiphanes norvegica* were sorted by sex (females have a conspicuous red thelycum), moult status (soft or hard) and, for females, whether gravid or not (denoted by the ovary's blue/grey colour through the carapace when gravid), while such an analysis was not possible for *Thysanoessa raschii*. All individuals were stored in the freezer at –20 °C in labelled plastic zip-lock bags, with a proportion set aside for additional biometric measurements. On occasion, *Calanus* were also removed from these tows, and processed as described for the WP-2 net samples (above). The sample remaining in the sieve was decanted into a glass Kilner jar (0.5 or 1 litre) with the aid of a plastic wash-bottle, and fixed in 4 % formalin in tap water (= 1.5 % formaldehyde). The contents of the two sets of WP-2 tows carried out in August 1999 were similarly fixed in 4 % formalin.

Back at DML, the frozen krill set aside for further biometric analysis were placed in labelled plastic weighboats and oven-dried at 60 °C for ~48 h. Given an optimum sample size for the Europa elemental analyser of ~300 μg C sample^{-1}, larger specimens needed to be subsampled. The dried individuals were therefore weighed, ground as finely as possible in a pestle and mortar, and up to four weighed subsamples of ~1 mg placed into separate tin capsules. The capsules were crushed and stored in vials as for the copepod samples (above). The formalin-fixed samples were drained and preserved in 70 % alcohol, having spent at least a month in fixative. All zooplankton species in the preserved samples taken by the 300 μm MOCNESS nets and 500 μm WP-2 net were identified and counted. The alcohol was drained by pouring through a 100 μm sieve, and the animals transferred to a Folsom plankton splitter containing fresh tap water. One or two splits were made, depending on the amount of material, with the sample being stirred with a random motion with a spoon before making the split. The final split was

decanted into a 250 ml conical flask and the volume made up to 250 ml with fresh tap water. The flask was shaken in a random fashion before removing a 5 ml aliquot with a stempel pipette. The contents of the pipette were transferred to a Bogorov counting tray, and all animals identified and counted under a binocular microscope (Olympus SZX 9 with 10× magnification eyepieces). Up to three aliquots were taken with the pipette so that between 100 and 200 individuals of each species were counted per split. For *Calanus* (stages CII to adult), if less than 100 individuals in total had been counted after three aliquots, a larger sample was taken from the conical flask. At least 100 individuals were counted, and the percentage frequency of each stage from this count used to convert the original total counts from the stempel pipette. The preserved samples taken by the 2 mm MOCNESS nets were used for the identification and enumeration of *M. norvegica* and *T. raschii*.

4.2.7 Starvation experiments conducted on *Calanus* adult females

In August 1999 and March 2000, adult female *Calanus* were collected at dawn (as described in section 4.2.5), when they were predicted to be at their daily maximum dry, carbon and nitrogen weight, and incubated in 0.45 μm-filtered seawater. This was to follow the time-course of body-weight loss in a starved individual, thus providing a baseline for the measurements of individuals taken directly in the field at different times during the diel cycle. As before, the contents of the cod end were poured through a 5 cm-diameter, 500 μm mesh sieve in the onboard lab, and placed under a binocular microscope in a petri dish containing just enough filtered seawater to cover the bottom of the mesh. Approximately 10 to 20 individuals were transferred with forceps into each of several 250 ml mesh-bottomed (500 μm) plastic tri-pour beakers sitting in 400 ml plastic tri-pour beakers containing 0.45 μm-filtered seawater. The mesh bottom allowed

faecal pellets to pass through so that no coprophagy by the study animals could occur. These beakers had been refrigerated to the approximate water temperature to be found at that particular time of year at the daytime residence depth of the migrants. The number of beakers filled was dependent on the abundance of animals in the net tow. The labelled beakers were returned to the fridge, where they were incubated in the dark for a given length of time.

At each designated time-point, a beaker was removed from the fridge and the copepods processed as described in section 4.2.5. In August 1999, two separate experiments were carried out, with measurements of prosome length (individuals) and carbon and nitrogen weight (samples) being made at time-points of 0, 1, 2, 4 h and either 6 h (1st experiment) or 9 h (2nd experiment). In March 2000, measurements of prosome length and gut fullness (individuals) and carbon and nitrogen weight (samples) were made at 0, 0.5, 1.5 and 4.5 h. In all three experiments, between one and four individuals were placed into pre-weighed Elemental Microanalysis Ltd. tin capsules (8 mm × 5 mm), frozen at –20 °C in 96-well microtitre plates and returned to shore for oven drying and elemental analysis.

4.2.8 Acoustic sampling

An RD Instruments (RDI) 300 kHz Workhorse (Broadband*) acoustic Doppler current profiler (ADCP) was attached to the moored instrument array in Inchmarnock Water in upward-looking mode at 110 m (see Table 4.1 for the dates of the mooring deployments). The instrument was set to collect data from 15 depth 'bins' of 8 m depth, and to fire pulses of sound ('pings') 12 times every 2 min. The resulting standard

* In this instance, the ADCP would have produced 'sweeps' between approximately 75 and 400 kHz, with the mean frequency being at 300 kHz.

deviation of velocity measurements was 0.6 cm s^{-1}. At 300 kHz, the wavelength of a sound pulse will range from a minimum of 4.82 mm at depth (110 m, 7.6 °C, 33.6 psu) to a maximum of 4.89 mm at the surface (0 m, 15.2 °C, 31.9 psu) (calculated from Equations 3.3 and 3.4). With a sound absorption rate of 0.062 dB m^{-1}, the range of this instrument under battery power was 120 m (under AC power the range would have been 240 m).

The maximum depth of the data was therefore set by the depth of the instrument (110 m) and the acoustic blanking region, which is a 'blind spot' just in front of the instrument (4 m in this case). The ADCP produces this 'blank (delay) beyond transmit' in order to give the electronics time to recover after transmitting before receiving the backscatter signal. Furthermore, data do not extend to the sea surface in this instance due to the final bin extending beyond the sea surface, and the penultimate bin (2-10 m) being influenced by surface reflection. The mid-points of the 12 depth-bins for which data were extracted were therefore at 14, 22, 30, 38, 46, 54, 62, 70, 78, 86, 94 and 102 m. The echo counts (a function of the RSSI parameter, see section 3.4.2) were converted to decibels (dB) using the calculations laid out by Deines (1999), who explained, "relating the intensity of an echo to the scatterers in the water requires knowledge of several variables: the power transmitted into the water, the acoustic characteristics of the transducer and the resulting acoustic beam, the power attenuation caused by propagation losses (including absorption and beam spreading), and the properties of the receivers". The dB values thus produced are referred to as the 'mean volume backscatter strength' (*MVBS*, or, more commonly, *Sv*). This is the logarithm of the mean cross-sectional area of backscattering particles within a mean volume of water. Due to beam spreading, this mean sampled volume increases with increasing distance from the instrument. However, the instrument automatically compensates for this, meaning the

Sv values from different depths are directly comparable with each other. The Doppler-derived vertical velocities (*VV*) were also representative of this mean volume, and were expressed in mm s^{-1}. The *Sv* and *VV* data were considered with respect to the net samples in order to determine behavioural patterns.

4.3 Results

4.3.1 The physical environment: CTD and light data

Seasonal changes in temperature, salinity and dissolved oxygen

Figure 4.5 shows the profiles (0-155 m) of temperature, salinity, dissolved oxygen (from October onwards) and density in Inchmarnock Water during each ship visit. On July 1st, the water column was well stratified ($\Delta\sigma_t$ = 1.2 kg m^{-3})*, with a warmer (12.0 °C), fresher (33.0 psu) surface mixed layer overlying a cooler (9.0 °C), more saline (33.6 psu) deep water layer. A thermocline was present between 18 and 30 m, through which the temperature decreased from 11.5 to 10.0 °C (-0.1 °C m^{-1}). A halocline was present between 16 and 20 m, through which the salinity increased from 33.0 to 33.3 psu (+0.1 psu m^{-1}). These observations indicate that stratification at this time was a product of both solar heating at the surface and the input of freshwater from local discharges.

By August 10th, the water column had become even more strongly stratified ($\Delta\sigma_t$ = 2.5 kg m^{-3}). The surface layer had warmed by 3.0 °C and freshened by 1.0 psu since July, indicating the persistence of warm weather and freshwater input. The deep water layer had also warmed by 1 °C and freshened by 0.1 psu, indicating that a degree of vertical mixing had been occurring from the surface layer (most likely the result of

* σ_t, a measure of density, is calculated as a function of temperature and salinity. The $\Delta\sigma_t$ values presented here represent the difference between σ_t at the surface (0 m) and σ_t at depth (120 m).

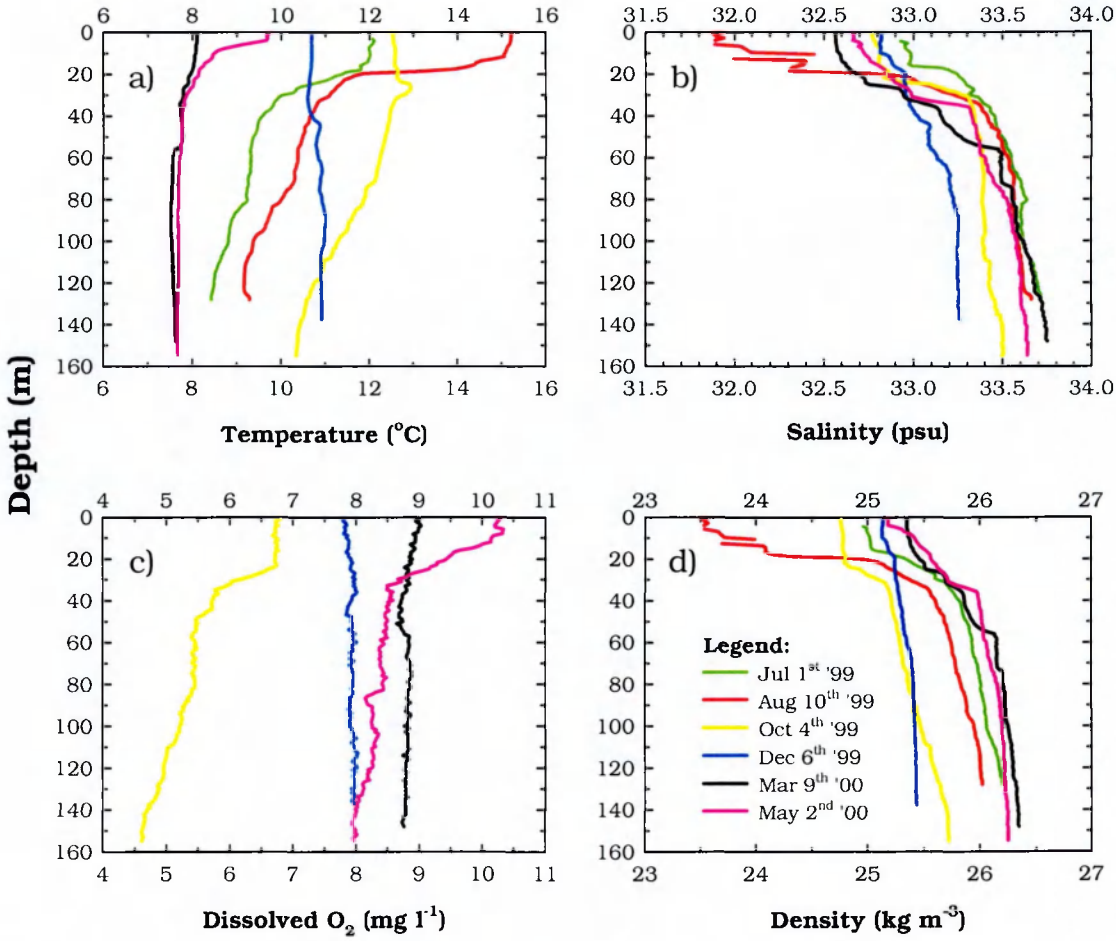

Figure 4.5 CTD-measured profiles of a) temperature, b) salinity, c) dissolved oxygen, and d) density (σ_t) in Inchmarnock Water for the period July 1999 to May 2000. Individual readings were made at 1 m depth intervals. Processed data courtesy of C.R. Griffiths at DML.

internal waves generated by the barotropic tide at the sill). The surface mixed layer had also shallowed since July. A thermocline was now present between 12 and 23 m, in which the temperature dropped from 15.0 to 11.5 °C (-0.3 °C m^{-1}), while a halocline was present between ~10 and 20 m. The exact salinities within this halocline were unknown due to problems with the conductivity cell of the CTD at this point.

By October 4th, the summer stratification began to break down ($\Delta\sigma_t$ = 1.0 kg m^{-3}). The surface mixed layer, which had now deepened to ~25 m, was 2.5 °C cooler and 0.9 psu more saline than in August. Furthermore, dissolved oxygen was highest and relatively uniform in this surface layer, at a mean level of 6.7 mg l^{-1}. The marked

deepening and cooling of the surface layer was most likely a product of cooler air temperatures and increasing wind-stress associated with the seasonal changes in both sunshine levels and weather conditions. The lower relative increase in the surface salinity also indicates that freshwater buoyancy inputs were still helping to maintain at least some stratification. This is supported by the presence of a halocline between 24 and 32 m, in which salinity increased from 33.0 to 33.3 psu (+0.1 psu m^{-1}). The deep water layer, however, was 1.0 °C warmer and 0.1 psu fresher than in August, indicating the continuing vertical mixing of water from the surface layer. It is likely that the mixing contribution from wind-stress was becoming more important, and that from internal waves less important, as the winter progressed and the pycnocline continued to weaken (since the magnitude of internal waves is positively correlated to the magnitude of the pycnocline). A noticeable temperature inversion between 25 and 35 m was also indicative of this increase in vertical mixing, as packets of warmer, less dense water were entrained to depth. The presence of a relatively strong oxycline at corresponding depths to the halocline, however, and the lower levels of dissolved oxygen in the deep water layer (5.3 mg l^{-1}), were further evidence that complete vertical mixing had not yet occurred.

By December 6th, the water column (0-138 m) was completely mixed in terms of temperature, dissolved oxygen and density ($\Delta\sigma_t$ <0.1 kg m^{-3}). Lower salinities were still evident at the surface, however, indicating the persistence of freshwater input. The mean temperature of the whole water column (0-138 m) was now 11.0 °C. While this meant that the surface layer was nearly 2.0 °C cooler than in October, the deeper layer (below ~120 m) was now actually warmer by ~0.5 °C. Dissolved oxygen levels had increased since October, most probably due to increased surface wave action associated with the seasonally unsettled weather. The complete vertical mixing meant that levels

remained relatively uniform with depth, at a mean value of 8.0 mg l^{-1}.

By March 9th, the water column had begun to restratify ($\Delta\sigma_t$ = 1.0 kg m^{-3}). Temperature decreased gradually with depth, from 8.1 °C at the surface to 7.7 °C at 35 m (<-0.1 °C m^{-1}). Below 35 m, the temperature remained relatively uniform, at around 7.6 °C. At 3.0 to 3.5 °C cooler than in December, the water column was now around its annual temperature minimum. A relatively low salinity (32.6 psu) surface mixed layer extended to ~10 m. Below 10 m, the salinity increased relatively rapidly to 33.5 psu at 60 m (+0.02 psu m^{-2}), after which values increased more gradually towards 33.8 psu at 148 m. The more marked changes in salinity, and their closer correspondence to the σ_t profile, suggest that freshwater rather than thermal inputs were responsible for the onset of stratification at this time. Levels of dissolved oxygen had increased since December (mean = 8.8 mg l^{-1}), probably due to the persistence of surface wave action throughout the winter period. The fact that levels were relatively uniform throughout the water column is further evidence that the restratification process was still in its early stages.

By May 2nd, the water column had become marginally more stratified ($\Delta\sigma_t$ = 1.1 kg m^{-3}). In the top 3 m, temperature was uniform at ~9.7 °C. A thermocline was now present between 3 m and ~30 m, through which the temperature decreased from 9.7 °C to 7.9 °C (-0.1 °C m^{-1}). Below 30 m, values were almost identical to those in March, at a mean of 7.7 °C. Surface salinity had increased marginally since March, increasing from 32.7 psu at the surface to 33.3 psu at 37 m (+0.02 psu m^{-2}). Below 37 m, salinity increased gradually towards a value of 33.6 psu at 155 m. The increase in both surface temperature and salinity since March indicated that thermal buoyancy inputs were beginning to assume a greater importance. Dissolved oxygen levels at the surface were near their annual maximum, decreasing rapidly from 10.3 mg l^{-1} at the surface to 8.7 mg l^{-1} at 30 m. Below 30 m, levels decreased more gradually towards 8.0 mg l^{-1} at 155 m.

Diel and seasonal changes in light

Figure 4.6 shows the diel changes in both absolute light intensity (Watts m^{-2}) and the relative change in light intensity (min^{-1}) during each ship-visit. Relative change was calculated as:

$$\text{Relative change } (\text{min}^{-1}) = \frac{\Delta I}{\bar{I}} dt$$

Equation 4.1

where I is the absolute light intensity at time t. 'First-light' and 'last-light' were defined as the times at which the absolute light levels systematically increased from, or decreased to, their average nighttime values. Table 4.3 summarises the day lengths (from first- to last-light) and absolute light levels during each sampling mission. The longest days were encountered during the Jun'99, Aug'99 and May'00 ship visits (20.7, 17.8 and 18.9 h, respectively), and light levels were relatively high (max. = 365, 800

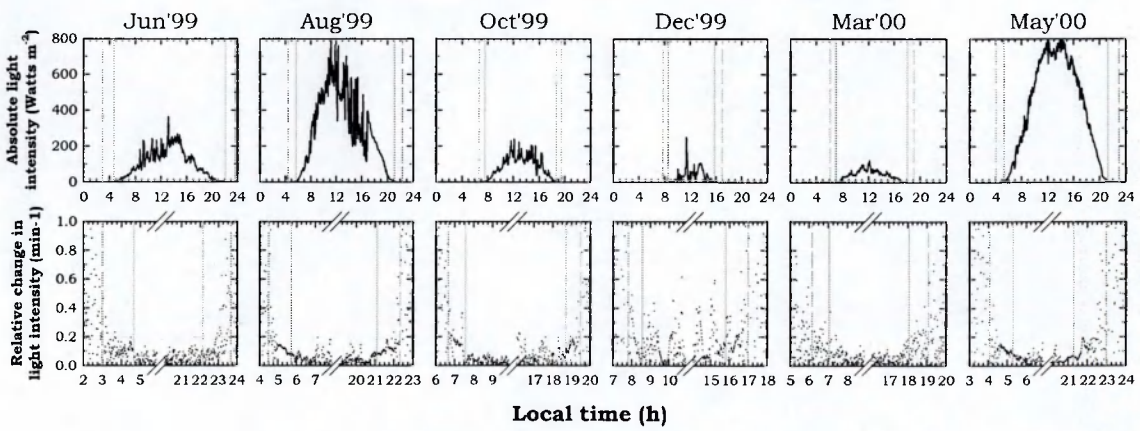

Figure 4.6 Daily light levels (Watts m^{-2}) and the relative change in light intensity (min^{-1}) (see Equation 4.1) during the period June 1999 to May 2000 at a site ~10km from Inchmarnock Water. The data shown represent an 'average day'. This is the average of the light levels on each of the days during which WP-2 net tows were made. All times are local. Data courtesy of G.A. Tarling at DML.

Date	Irradiance (W m^{-2})			Local time (hh:mm)				
	Night mean	Mid-day	Day max	First light	Sun-rise	Sun-set	Last light	Day length
Jun'99	0.001	182	365	03:00	04:40	22:09	23:40	20:40
Aug'99	0.001	670	802	04:30	05:44	21:07	22:20	17:50
Oct'99	0.001	137	239	06:40	07:35	18:42	19:30	12:50
Dec'99	0.001	50	249	07:50	08:35	15:49	17:00	09:10
Mar'00	0.003	100	121	06:10	07:03	18:04	19:05	12:55
May'00	0.001	746	809	04:05	05:18	21:18	23:00	18:55

Table 4.3 Synopsis of daily light levels during the period June 1999 to May 2000 at a site ~10km from Inchmarnock Water. The values are computed from an 'average day'. 'First-light' and 'last-light' refer to the time at which light levels either systematically increased from, or decreased to, their mean nighttime values. 'Official sunrise' and 'Official sunset' signify the time at which the top of the sun was exactly in line with the horizon (obtained for the study site location from the URL: http://aa.usno.navy.mil/data).

and 810 W m^{-2} respectively). The shortest days were encountered during the Oct'99, Dec'99 and Mar'00 ship visits (12.8, 9.2 and 12.9 h respectively), and light levels were relatively low (max. = 240, 250 and 120 W m^{-2} respectively). The greatest rates of relative change occurred around first- and last-light during each sampling mission.

4.3.2 Primary production: measurements of chlorophyll a

Comparison between measurement types

Figure 4.7a shows the levels of SeaWiFS-derived chlorophyll *a* in the surface waters of Inchmarnock Water for the period January 1999 to December 2000. Working with the same dataset, Tarling *et al.* (2002) showed that, when concurrent chlorophyll *a* measurements from other sources were available, there were no significant differences between the SeaWiFS, water bottle or fluorometer data (Kruskal-Wallis: $P < 0.05$, see Table 4.4). The use of SeaWiFS data to assess the levels of surface chlorophyll *a* over the study period was therefore considered justifiable.

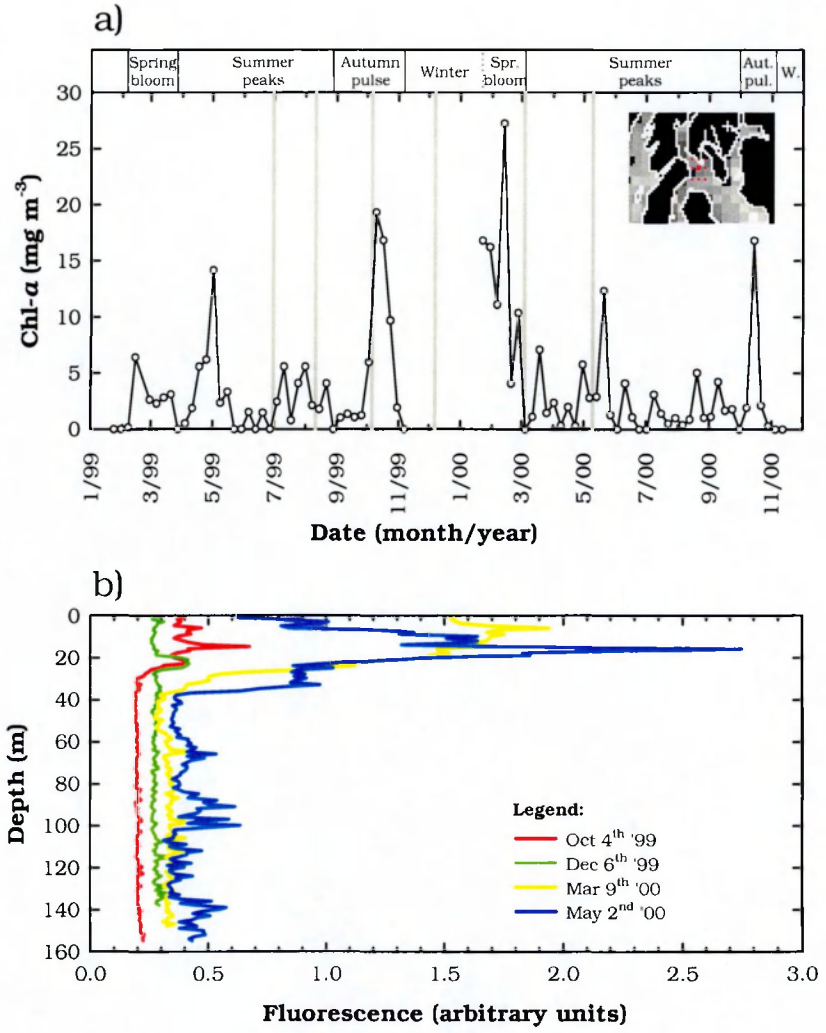

Figure 4.7 Levels of chlorophyll *a* in Inchmarnock Water. a) Surface chlorophyll *a* levels during the period January 1999 to December 2000, calculated from SeaWiFS for a 4 km pixel centred on 55.80 °N, 5.25 °W (see inset). Grey lines show the dates of ship visits. Data courtesy of P. Miller at PML. b) Fluorescence profiles from CTD casts during the period October 1999 to May 2000. Data courtesy of C.R. Griffiths at DML.

Seasonal changes in SeaWiFS-derived surface chlorophyll a

The SeaWiFS-derived chlorophyll *a* levels (Figure 4.7a) were used to interpret the seasonal cycles of phytoplankton and primary production during the present study. These interpretations were based on information from previous investigations such as those of Hannah & Boney (1983) and Tett *et al.* (1986) (see section 4.1.3).

In 1999, the "spring bloom/post-bloom" phase appeared to take place between February 7th and March 28th, during which chlorophyll *a* levels reached a maximum of 6.4 mg m^{-3}. This was followed by a prolonged "summer period" of periodic peaks in chlorophyll *a*, which appeared to continue right through until August 29th. The largest peak during this period reached 14.1 mg m^{-3}. An "autumn pulse" was apparent between August 29th and November 7th, during which chlorophyll *a* levels reached 19.3 mg m^{-3}. Data are unfortunately unavailable for the ensuing 1999/2000 winter period. When they do become available again on January 23rd 2000, it appears that the spring bloom had already started. This bloom continued until March 5th, during which period the chlorophyll *a* levels reached a maximum of 27.2 mg m^{-3}, over four times greater than the previous year's spring bloom. Another prolonged period of summer peaks followed which lasted until October 1st, a full month later than the previous year. The largest peak during this period was similar to 1999, reaching 12.3 mg m^{-3}. An "autumn pulse" was apparent between October 1st and November 5th, during which chlorophyll *a* levels, at a maximum of 16.8 mg m^{-3}, were of a similar magnitude to the previous year. As in 1999, data are again unavailable for a portion of the 2000/2001 winter period.

	Chl *a* (mg m^{-3}) measured by:		
Date	SeaWiFS	Bottle samples	Fluorometer
June 24 –26, 1999	0.35±0.77	0.83±0.44	-
August 23 – 28, 1999	1.81±2.53	-	1.79±0.67

Table 4.4 A comparison of the levels of chlorophyll *a* (±1SD) in the surface waters of Inchmarnock Water as determined by three separate methods: SeaWiFS ocean colour satellite, 2 m NIO bottle collections followed by HPLC analysis, and moored fluorometer at 1.5 m depth. No significant differences were found between concurrent data (Mann-Whitney U-test: $P < 0.05$) (after Tarling *et al.*, 2002).

Vertical fluorescence profiles

CTD-derived vertical fluorescence profiles (Figure 4.7b) showed that the levels of chlorophyll *a* were consistently higher in the surface layer of the water column. Furthermore, the depth at which the values fell to a uniform minimum coincided well with the bottom of the observed pycnocline (when present), suggesting that this feature was acting to trap the bulk of the phytoplankton cells in the surface layers. In October 1999, chlorophyll *a* levels peaked at 15 m, and reached a minimum at 30 m. In December 1999, values were generally low throughout the water column, although a definite increase was observed between 20 m and 30 m depth, possibly due to a gathering of senescent cells in this depth stratum. In March 2000, the chlorophyll maximum occurred close to the surface at 6 m, and levels reached a minimum at 40 m. In May 2000, the chlorophyll maximum occurred at 16 m, with levels again falling to a minimum at 40 m. Chlorophyll *a* values at depth, while remaining uniformly low from the base of the pycnocline (when present), showed a general increase from October through to May. One might ascribe the increase from October to December to the increase in vertical mixing implied by the hydrographic data (see section 4.3.1). Similarly, vertical mixing processes in March and May could also have been responsible for removing phytoplankton cells from the surface, although senescence, flocculation and the passive sinking of larger aggregates were other potential factors.

4.3.3 Secondary production: mesozooplankton dynamics

Net catch data

The mesozooplankton community: seasonal changes and vertical migration

Table 4.5 shows the species composition and concentration (ind. m^{-2}, 0-130 m) of all net-caught mesozooplankton (>300 μm). In the spring (May), the zooplankton

community was numerically dominated by the cladoceran *Evadne nordmanni* and the copepods *Temora longicornis*, *Calanus* and *Paracalanus/Pseudocalanus*. The summer community (June-August) was overwhelmingly dominated by the copepod *Acartia clausi*, although *Calanus* assumed an increasing importance as the summer progressed. By the autumn (October), *Calanus* was by far the most numerous species. Composed mostly of stage CV, *Calanus* continued to dominate throughout the winter (December-March).

Analysis of the depth-discrete net-catch data suggested that the most significant migrators (in terms of amplitude) in Inchmarnock Water during the course of this study were *Calanus* stages CIV, CV and adult females (Figure 4.8), *Meganyctiphanes norvegica* adults and juveniles, and *Thysanoessa raschii* (developmental stages undefined) (Figure 4.9). However, a variety of other taxa, namely *Paracalanus/Pseudocalanus*, cladocera, furciliae and calyptopes, *Centropages* spp. and decapod larvae also showed noticeable day/night changes in vertical distribution at various times during the year (Figure 4.10).

Calanus: population dynamics

The demographics of the *Calanus* population in Inchmarnock Water were inferred from the net-catch data presented in Table 4.5. In June 1999, the population was dominated by stages CIV, CV, and adult females. The lower numbers of younger stages indicated that spawning had not occurred recently.

By August 1999, the population had more than doubled in numbers and was now dominated by stage CV. While still constituting only a small percentage of the population, the 23-fold increase in the concentration of stage CII indicated that spawning had occurred since July.

	Concentration (ind. m^{-2}, 0-130 m)						
	Jun'99	Aug'99	Oct'99	Dec'99	Jan'00	Mar'00	May'00
Copepods							
Acartia clausi	60,000	3650	2350	250	50	25	900
Aetideus armatus ♀	0	1700	800	25	75	100	0
Calanus CII	7	170	80	0	0	15	1200
CIII	180	370	200	8	6	40	1800
CIV	330	700	575	35	20	60	2200
CV	400	1400	4000	600	90	40	1400
♀	400	500	290	90	60	20	500
♂	80	90	50	9	25	5	250
Centropages hamatus	650	0	0	0	0	0	0
C. typicus	0	75	50	2	<1	2	100
Euchaeta norvegica ♀	0	0	0	0	0	2	0
Metridia lucens ♀	0	0	0	15	3	2	5
Microcalanus sp.	0	5	0	0	0	0	0
Oithona spp.	<1	7	0	0	0	<1	0
Paracalanus/Pseudocalanus	5750	65	400	<1	0	5	7000
Temora longicornis	1200	15	25	0	0	3	13,300
Krill							
M. norvegica adults	6	7	10	5	n/a	5	1
juveniles	4	20	35	5	n/a	<1	<1
Thysanoessa raschii	20	130	50	1	n/a	<1	<1
Eggs	0	0	0	0	0	4	0
Nauplii	0	35	0	0	0	0	20
Calyptopes	0	85	0	0	0	<1	150
Furciliae	0	110	0	0	0	0	150
Cladocera							
Evadne spp.	5200	100	8	0	0	5	17,000
Podon spp.	80	300	0	0	0	2	35
Other							
Cirripede nauplii	1200	<1	0	0	0	0	0
Decapod larvae	40	70	0	3	7	100	60
Echinoderm larvae	0	0	0	0	0	4	0
Fish eggs & larvae	5000	275	0	0	0	400	900
Fish juveniles	0	0	0	<1	<1	<1	0
Medusae	0	30	0	0	0	<1	0
Molluscan larvae	0	1	0	0	0	<1	0
Oikopleura sp.	0	70	0	0	0	0	0
Polychaete larvae	0	1	0	4	1	<1	0
Sagitta elegans	4	25	100	25	2	3	9

Table 4.5 The total concentration of all net-caught mesozooplankton (>300 μm) in Inchmarnock Water (ind. m^{-2}, 0-130 m) during the period June 1999 to May 2000.

By October 1999, the population had nearly doubled again. This was entirely due to a three-fold increase in the numbers of stage CV, which now made up 75 % of the population. The continued presence of stages CII and CIII suggests that a new generation may well have been spawned within the last few weeks.

By December 1999, the population had become dramatically reduced, with the lack of stage CII individuals indicating that breeding had not taken place since October.

By January 2000, the population had decreased further. Stage CV, in particular, appeared to have undergone severe reductions in numbers. Conversely, the numbers of adult males had increased almost 3-fold, suggesting that at least some of the reduction in the numbers of stage CV was due to their having moulted to adults.

The lowest concentrations were found in March 2000, although increases in the numbers of stages CII, CIII, and CIV indicated that a new generation had recently been produced.

The highest concentrations were found in May 2000, with the numbers having increased dramatically since March. The extremely high concentration of stage CII indicated that a major spawning had recently occurred. The relatively low numbers of adults suggests that these were the remains of a previous generation that was now dying off following copulation and spawning, while the relatively high numbers of stage CIV and CV hints that the population was beginning to be augmented by the next generation.

Calanus: vertical migration behaviour

The DVM behaviour of *Calanus* in Inchmarnock Water was inferred from the net-catch data presented in Figure 4.8. These data showed that, out of those stages that were identified and enumerated (i.e. stage CII to adults), stages CIV, CV and adult females performed the most marked vertical migrations in terms of amplitude.

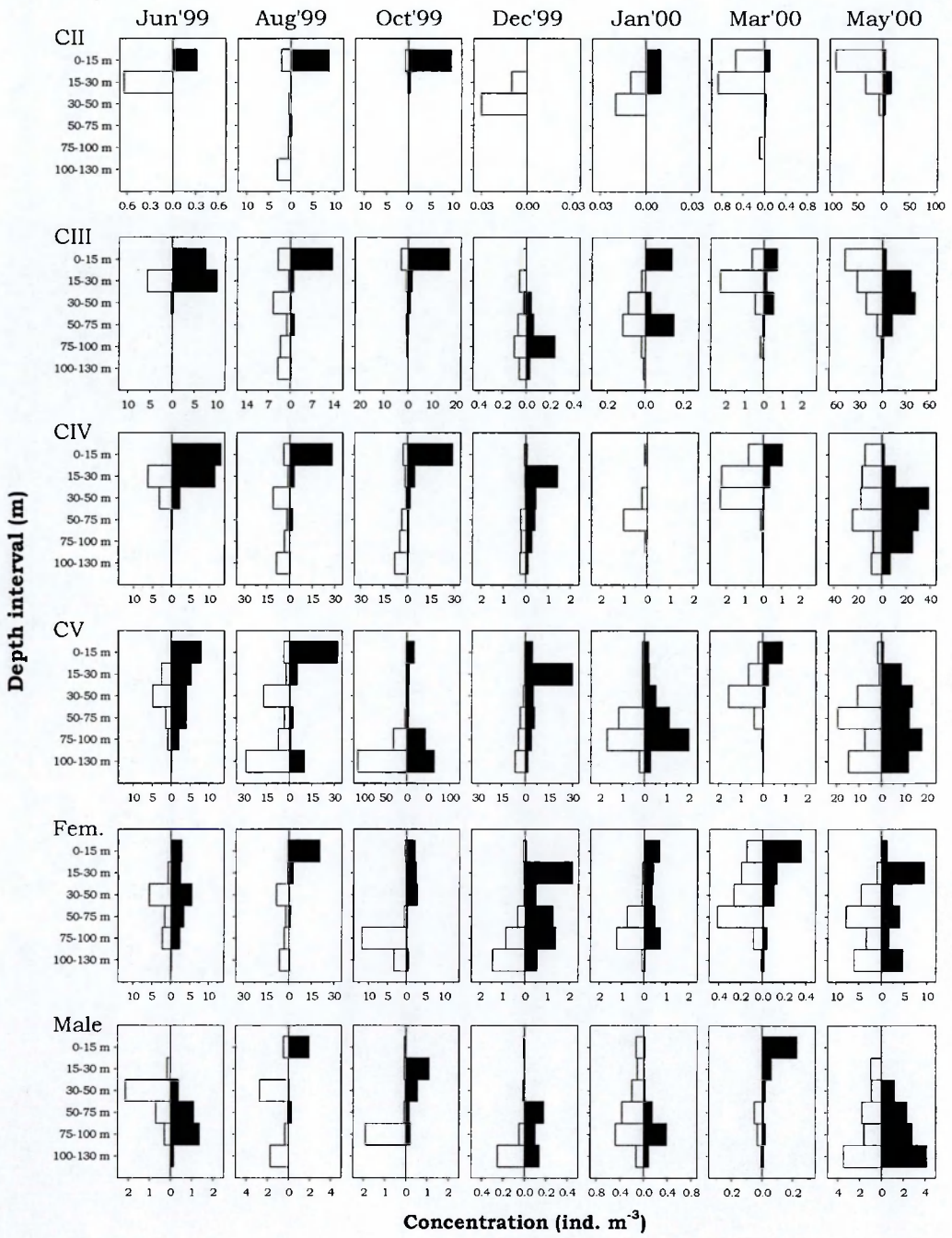

Figure 4.8 The vertical distribution and concentration of *Calanus* (stages CII to adult) in Inchmarnock Water by day (white bars) and by night (black bars) during the period June 1999 to May 2000. Note the changing *X*-axis (concentration) scale between each chart. All data are from MOCNESS-1 samples except those during August, when a 1 m WP-2 net was used.

In June 1999, these three stages showed a relatively weak pattern of DVM: where individuals were completely absent from the top 15 m by day (11:00 h), some individuals (up to 10 ind. m^{-3}) were present in this stratum at night (01:00 h). Stage CV

and adult females were found to depths of 100 m both day and night, while stage CIV were mostly found closer to the surface and extended to only 50 m depth. At least some of the *Calanus* population was therefore migrating through the pycnocline (18-30 m) on a daily basis at this time.

DVM behaviour was more marked in August 1999, with individuals from each stage being found almost exclusively (up to 30 ind. m^{-3}) in the top 20 m at night (22:00 h). In the day (12:00 h), stage CIV were fairly evenly distributed between the surface and 130 m. Stage CV were also distributed over this depth range, but showed concentration maxima in both the 40-60 m (15 ind. m^{-3}) and 100-130 m (30 ind. m^{-3}) depth strata. The presence of relatively high numbers at 100-130 m both day and night (8 to 30 ind. m^{-3}) suggests that a proportion of the stage CV stock was not migrating towards the surface every day. Adult females were almost completely absent from the top 40 m in the day, and therefore underwent the most extensive DVM. These observations suggest that significant fractions of these three stages were migrating through the marked pycnocline (12-23 m) on a daily basis at this time. An additional series of tows between 11:00 and 03:30 h showed that some of the *Calanus* population was actually reaching the top 20 m at least ~3 h prior to sunset, i.e. by ~18:40 h (Figure 4.11).

A marked change in the behaviour of stage CV was apparent in October 1999, with most individuals (up to ~100 ind. m^{-3}) now remaining below 75 m both day and night, and only a proportion (~20 ind. m^{-3}) migrating into the top 30 m at night. The bulk of the stage CIV and adult female stocks, on the other hand, continued to undertake a relatively marked pattern of DVM into the surface layer. As in August, the CIV stock was spread between 0 and 130 m in the day (13:30 h), moving mostly into the top 15 m at night (23:30 h) (~30 ind. m^{-3}), while the adult female stock was to be found entirely below 50 m in the day (up to ~10 ind. m^{-3}) and almost entirely above 50 m at night (~2

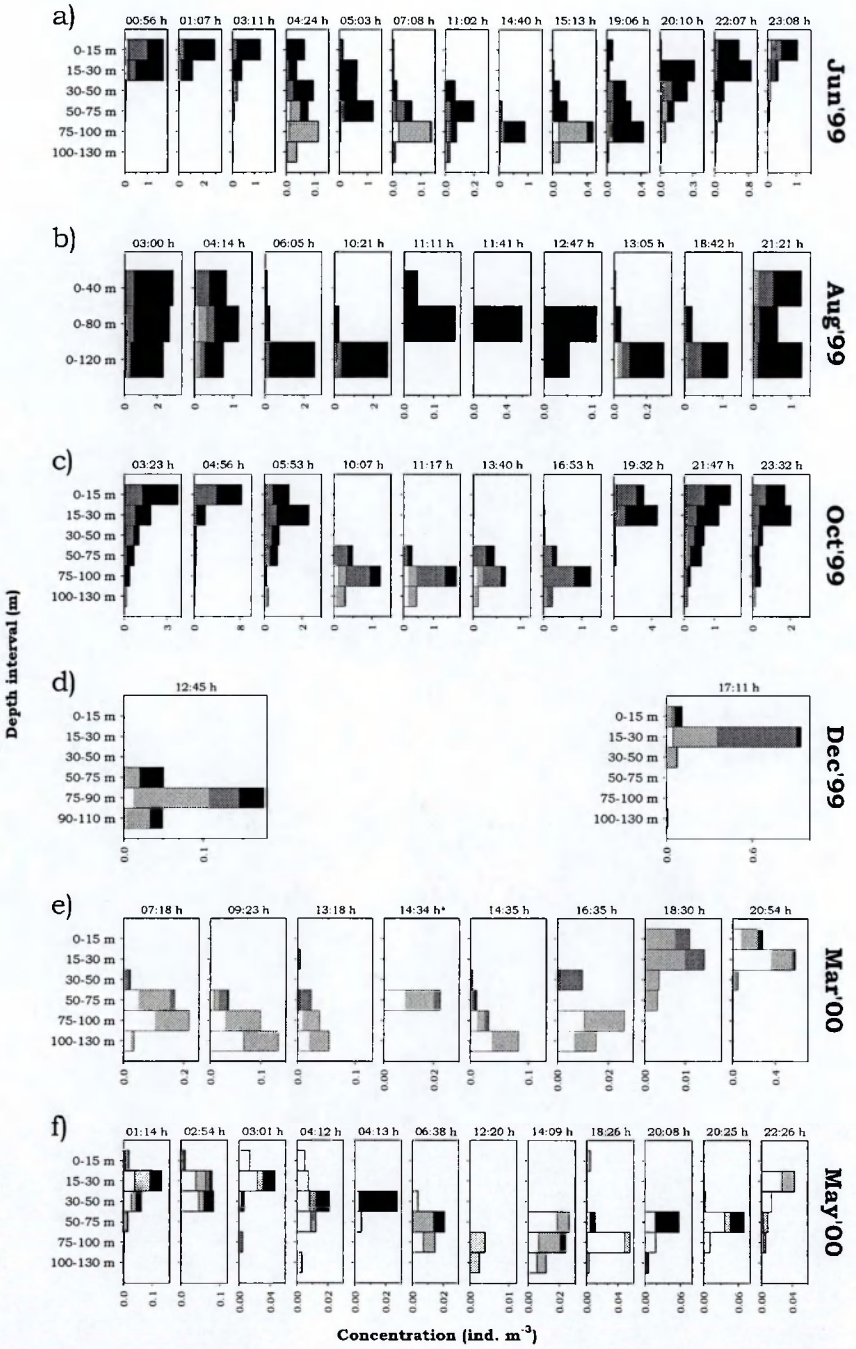

Figure 4.9 The vertical distribution and concentration of krill in Inchmarnock Water over the diel cycle during a) June/July 1999, b) August 1999, c) October 1999, d) December 1999, e) March 2000, and f) May 2000. The bars represent *M. norvegica* males (white), females (light grey) and juveniles (dark grey), and *T. raschii* (undefined developmental stages) (black). All times are local.

ind. m^{-3}). From this, it is likely that a noticeable proportion of the *Calanus* population was still migrating into and out of the ~25 m-deep surface mixed layer on a daily basis at this time.

By December 1999, a greater fraction of the stage CV stock had now resumed DVM. Stage CIV and adult females continued to migrate as before, although stage CIV did not appear to ascend as close to the surface, and a greater proportion of adult females were found at depth (>50 m) during the night. For each of these three stages, individuals were mainly found below 50 m during the day (12:30 h) (up to 7 ind. m^{-3}), and moved mostly into the 15-30 m layer at night (17:00 h) (up to 30 ind. m^{-3}). A small proportion also moved into the top 15 m (up to ~5 ind. m^{-3}). It is therefore likely that the bulk of the population, albeit strongly diminished at this time, was migrating between the 0-30 m depth interval and >50 m on a daily basis, although there was no pycnocline present across which they would have been moving.

In January 2000, while DVM still appeared to be occurring, the proportion of migrating individuals was reduced. Adult females were mostly found below 50 m by day (up to ~1 ind. m^{-3}), becoming spread between the surface and 130 m by night. The bulk of the stage CV stock remained below 50 m both day and night (up to 2 ind. m^{-3}), although some individuals (<1 ind. m^{-3}) moved into the top 30 m at night. Since no stage CIV individuals were caught at night, no diel movements could be inferred in this instance.

In March 2000, a fairly weak DVM signal was apparent in all three stages. While found from the surface to as much as 100 m in the day (11:30 h), there was a noticeable movement of individuals (up to 1 ind. m^{-3}) into the top 15 m at night (21:00 h). It is likely, therefore, that a proportion of the population was migrating through the weak pycnocline (10-30 m) on a daily basis at this time.

There was little evidence for DVM by any stage in May 2000, with individuals being found in relatively high numbers (up to 40 ind. m^{-3}) throughout the water column both night and day.

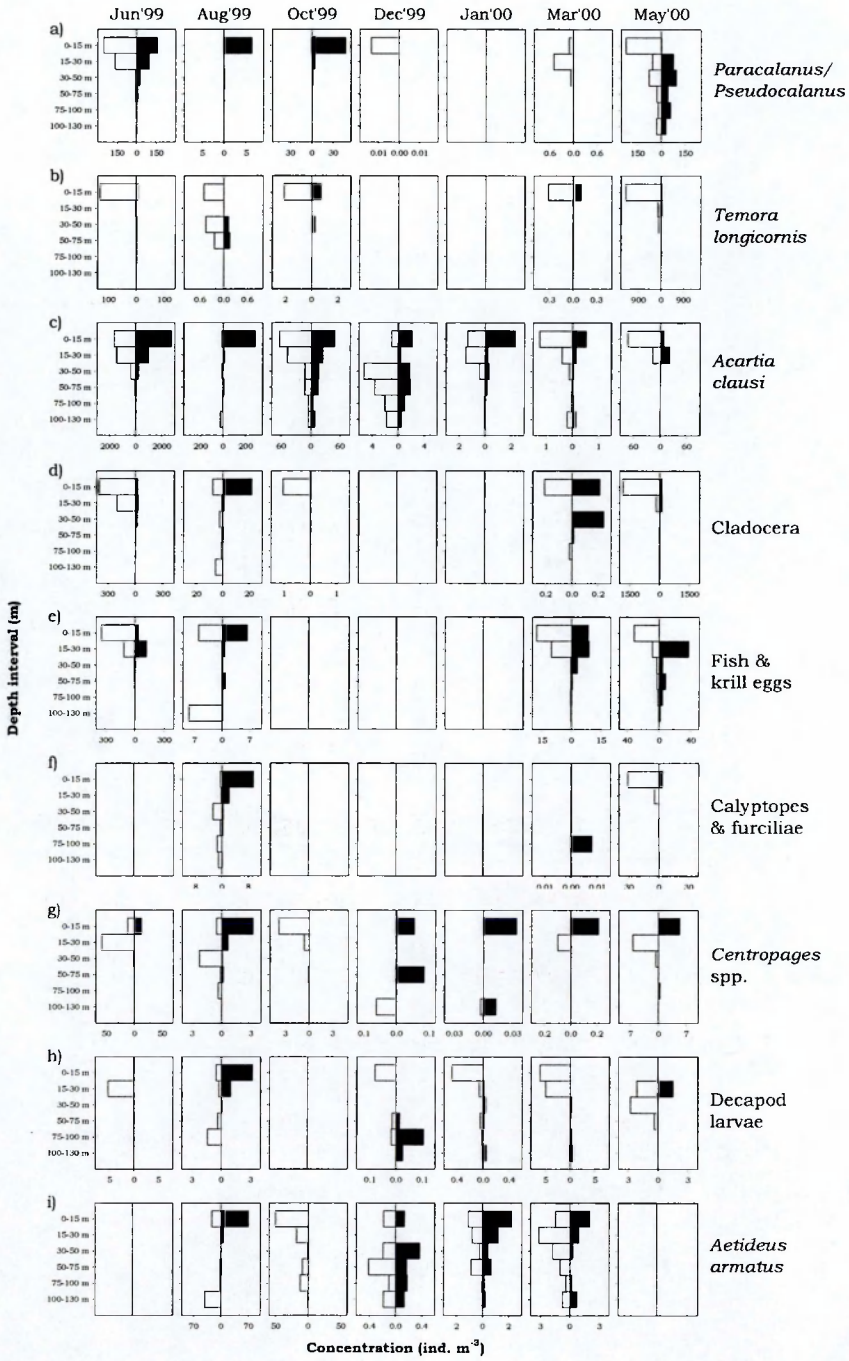

Figure 4.10 The vertical distribution and concentration of the nine most abundant mesozooplankton taxa (excluding *Calanus* and krill) in Inchmarnock Water by day (white bars) and by night (black bars) during the period June 1999 to May 2000. Blank graphs denote that no individuals were caught. Note the changing *X*-axis (concentration) scale between each chart. All data are from MOCNESS-1 samples, except those during August when a 1 m WP-2 net was used.

Krill: population dynamics

The demographics of the krill population in Inchmarnock Water were inferred from the

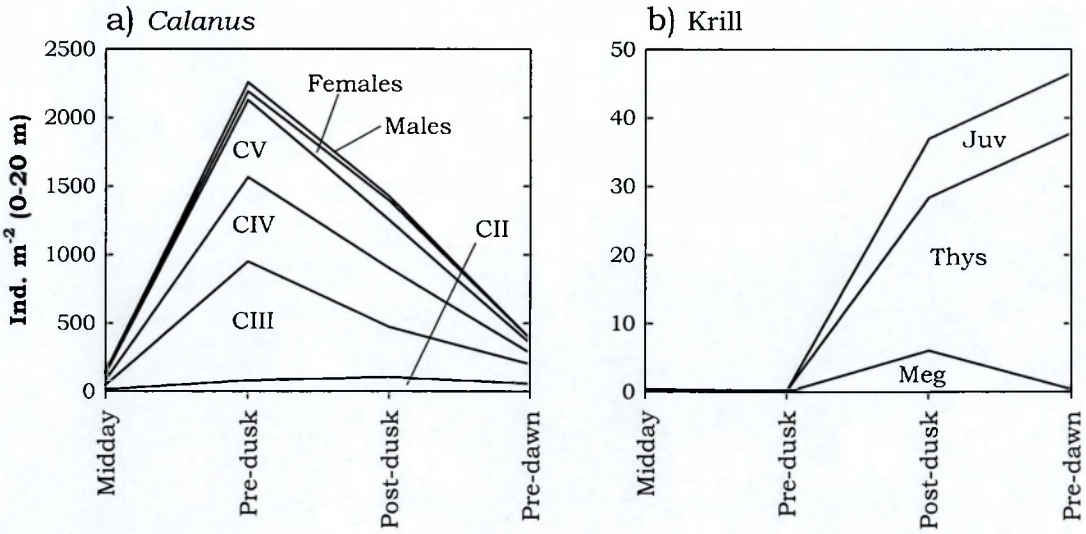

Figure 4.11 The concentration (ind. m^{-2}) of *Calanus* (stages CII to adult) and krill (*Meganyctiphanes norvegica* adults, *Thysanoessa raschii* adults, and undefined juveniles) in the top 20 m of the water column in Inchmarnock Water, from double-oblique MOCNESS tows made during four time-periods between 11:00 and 03:30 h in August 1999. Midday = 11:00 to 13:00 h. Pre-dusk = 18:40 to 19:00 h. Post-dusk = 21:20 to 21:40 h. Pre-dawn = 03:00 to 03:30 h.

net-catch data presented in Table 4.5. The occurrence of eggs in March 2000, and nauplii, calyptopes and furciliae in May 2000, indicated that spawning occurred in the spring. Furthermore, a peak of nauplii, calyptopes and furciliae in August 1999 indicated that a second spawning occurred in late summer. The increase in numbers of *M. norvegica* juveniles from July 1999 to October 1999 implies that the population had been recruited initially from the spring spawning, and then augmented further by the subsequent late-summer spawning. From October 1999 onwards, however, both the juvenile and adult populations were seen to decline with the onset of winter. The *T. raschii* population peaked slightly earlier, in August 1999, after which numbers then declined towards winter.

Krill: vertical migration behaviour

The DVM behaviour of *M. norvegica* and *T. raschii* in Inchmarnock Water was inferred

from the net-catch data presented in Figure 4.9. Both species showed evidence of marked, synchronous NDVM, with the most noticeable changes in vertical distribution corresponding with the changing light levels around dawn and dusk. In general, individuals resided in the 50 to 100 m depth interval by day (with overall concentrations up to ~2 ind. m^{-3} in October), and moved into the top 30 m at night (with overall concentrations up to ~8 ind. m^{-3} in October). There appeared to be a degree of variability in the vertical distribution of individuals and the timing of movements, both between and within species as well as between sampling months. For example, *M. norvegica* juveniles and *T. raschii* were often found closer to the surface than *M. norvegica* adults both day and night. In some cases (e.g. June/July 1999), it appeared that *M. norvegica* adults were spending less time in the surface layer at night than juveniles and *T. raschii*, although such direct comparisons were often hampered by the fact that only one group tended to dominate at any one time.

Acoustic data

The continuous nature of the data from the moored 300 kHz ADCP in Inchmarnock Water allowed diel and seasonal changes in both mean volume backscatter (*Sv*, in dB) and vertical velocities (*VV*, in mm s^{-1}) to be resolved in detail. However, day-to-day variability masked any underlying patterns, so days were combined to give an 'average-day' for each ship visit. In those instances when the instrument was not in the water concurrently with a ship visit (see Table 4.1), the nearest available 5 d period was chosen.

Table 4.6 shows the designated average-day time-periods and the corresponding dates of each ship visit. The average-day acoustic data are presented graphically as colour-filled contour plots (*Sv*: Figure 4.12, *VV*: Figure 4.13). The contours were

Ship visit	ADCP 'average day'
June 28 – July 2, 1999	June 28 – July 2, 1999
August 9 – 13, 1999	August 9 – 12, 1999
October 4 – 8, 1999	October 9 – 13, 1999
December 7, 1999	December 1 – 5, 1999
March 1 – 7, 2000	March 10 – 14, 2000
May 8 – 12, 2000	May 8 – 12, 2000

Table 4.6 The combined dates used to calculate the ADCP 'average-day' in Inchmarnock Water, compared with the dates of the ship visits.

generated using SigmaPlot for Windows version 6, which employed a default kriging algorithm to interpolate between the data-points (kriging involves a bilinear algorithm which places equal emphasis on all values immediately surrounding an empty pixel). Overall, *Sv* ranged from –47.84 to –95.31 dB, and *VV* from +210 to -345 mm s^{-1}.

Mean volume backscatter (Sv)

From June to December 1999, a distinct sound scattering layer (SSL) was consistently present between 50 and 90 m during the day (Figure 4.12a-d). This was consistent with the vertical distribution of some of the *Calanus* population and most of the krill population as inferred from the net catches (Figures 4.8 and 4.9, respectively). Maximum *Sv* values within the SSL were lower in June/July and December (–65 dB), and higher in August and October (–60 dB), mirroring the seasonal changes in the concentrations of both *Calanus* and krill (Table 4.5). In March and May 2000, when the net catches revealed low numbers of larger krill (Table 4.5), *Sv* did not exceed –70 dB (Figure 4.12e-f).

During every sampling month, including those when backscatter was relatively low (i.e. March and May 2000), the regions of increased backscatter exhibited diel changes in vertical distribution that implied NDVM behaviour. *Sv* values increased at 14 m

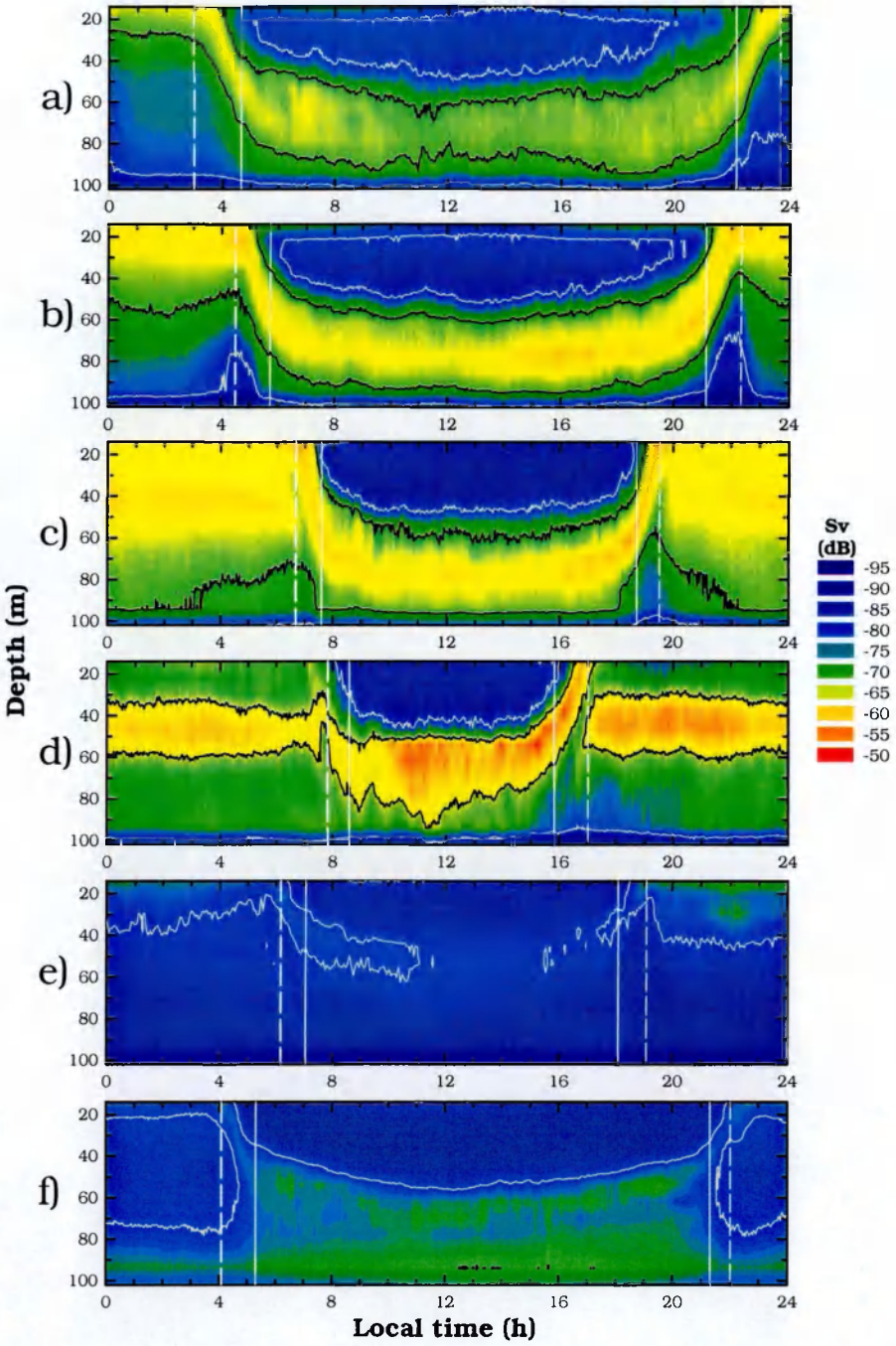

Figure 4.12 'Average-day' contour plots of mean volume backscatter (*Sv*, in dB), measured by an upward-looking 300 kHz ADCP moored at 110 m in Inchmarnock Water in a) Jun/Jul'99, b) Aug'99, c) Oct'99, d) Dec'99, e) Mar'00, and f) May'00. Contour lines are at –70 dB (black) and –80 dB (grey). Vertical lines show first and last light (broken lines), and sunrise and sunset (solid lines).

depth between 15 and 45 min after sunset (= dusk ascent), and decreased between 10 and 45 min before sunrise (= dawn descent) (Figure 4.14). In June/July and August 1999, the first arrival of the SSL at 14 m at dusk was preceded by a small but noticeable

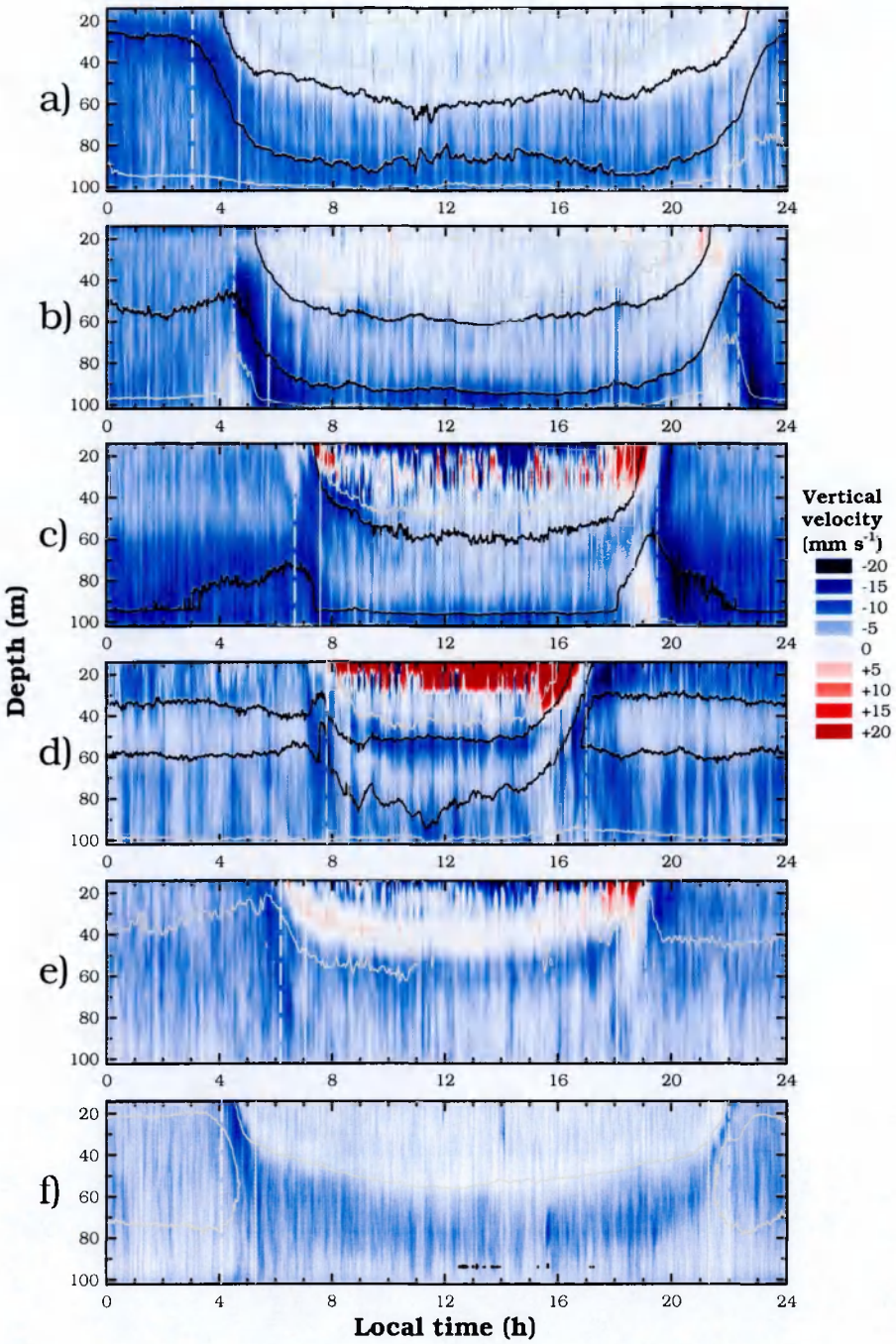

Figure 4.13 'Average-day' contour plots of Doppler-derived vertical velocity (*VV*, in mm s^{-1}), measured by an upward-looking 300 kHz ADCP moored at 110 m in Inchmarnock Water. a)-f) and sunrise/sunset as in Figure 4.12. Negative *VV* values represent downward movement, and *vice versa*. *Sv* contour lines are superimposed at –70 dB (black) and –80 dB (grey).

increase in *Sv* of ~5 dB (see red and green lines in Figure 4.14b). This increase began ~8 h ahead of the SSL in June/July, and ~7 h ahead in August (at ~14:00 h in both cases) and peaked at 20:00 h and 19:00 h, respectively. In October 1999, a small increase in *Sv*

began ~1 h before sunset (at ~17:30 h), after which levels increased markedly with the arrival of the SSL. The additional depth-integrated net tows in August 1999 (11:00 to 03:30 h) showed that *Calanus* could have been responsible for this pre-dusk ascent signature (Figure 4.11).

Another pattern of behaviour was also apparent from the acoustic data. From June to October 1999, *Sv* levels decreased in the 50-90 m layer following the dusk ascent of the SSL, but began to recover within ~1 h as backscattering particles dispersed from the surface to deeper water (Figures 4.12a-c and 4.15b). This post-dusk downward dispersion, or "midnight sinking" (after Cushing, 1951), became deeper and more marked over this period. In December 1999, midnight sinking was apparent from a post-dusk decrease in *Sv* at 14 m, but the lack of an accompanying increase in the *Sv* at 62 m indicated that midnight sinking did not extend deeper than 62 m at this time. The net-catch data (Figures 4.8 and 4.9) indicated that this pattern of midnight sinking was most likely to have been due to *Calanus* alone in June/July, August and December 1999, but that krill were almost definitely also involved in October. No such behaviour was

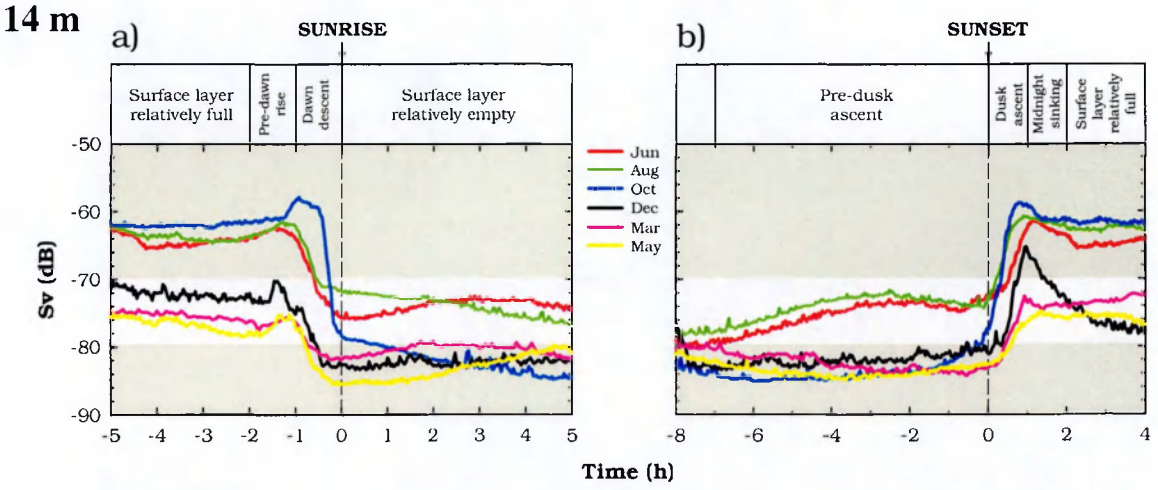

Figure 4.14 Changes in mean volume backscatter (*Sv*, in dB) at 14 m depth around a) sunrise, and b) sunset, in Inchmarnock Water during the period June 1999 to May 2000. The shaded areas represent the designated boundaries between "high", "medium" and "low" backscatter (see section 4.4.2).

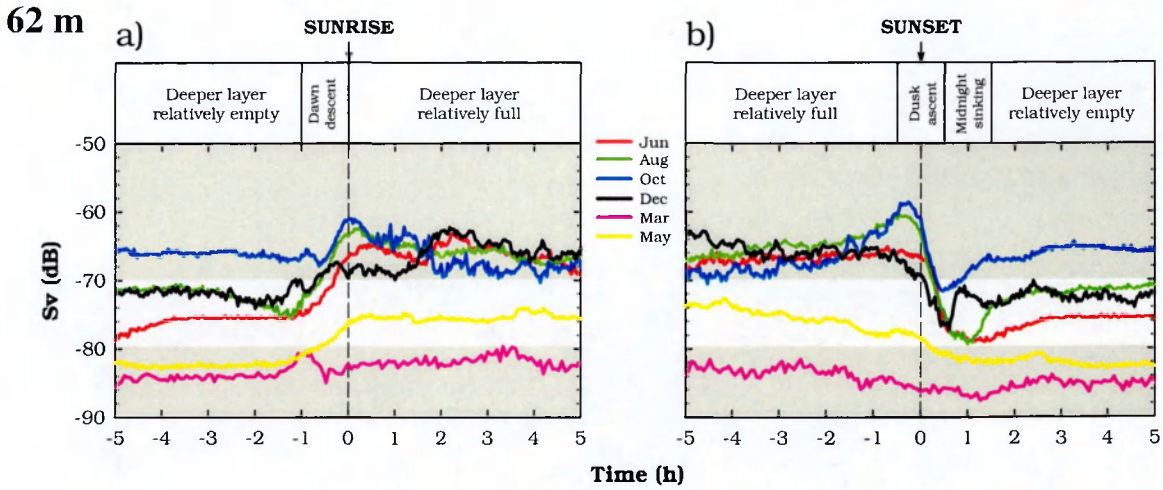

Figure 4.15 Changes in mean volume backscatter (*Sv*, in dB) at 62 m depth around a) sunrise, and b) sunset, in Inchmarnock Water during the period June 1999 to May 2000. The shaded areas represent the designated boundaries between "high", "medium" and "low" backscatter (see section 4.4.2).

observed acoustically in either March or May 2000.

Doppler-derived vertical velocity (VV)

The Doppler-derived *VV* vectors were predominantly downward during most stages of the diel cycle in all sampling months, and upward vectors were observed infrequently (Figure 4.13). Table 4.7 shows that the magnitude of this downward bias, or 'anomaly', was not consistent, but rather changed seasonally, implying an environmental rather than an instrumental causation. The *VV* data plotted in Figure 4.13 should therefore be viewed in relative terms as being either 'less negative' or 'more negative'. Furthermore, it was thought likely that *VV* values >50 mm s^{-1} (either up or down), most of which were found in the near-surface bins during the day in winter, were suspect and should therefore be ignored. These points are discussed further in section 4.4.2.

In general, the strongest *VV* corresponded to vertical movements of biomass as indicated by the *Sv* data. However, these did not always coincide with the location of the SSL. Strong negative *VV* values were observed around dawn in every sampling

Date	mm s^{-1}
June '99	-7
July '99	-8
Aug'99	-9
Sep'99	-10
Oct'99	-10
Nov'99	-8
Dec'99	-7
Jan'00	-6
Feb'00	-4
Mar'00	-4
Apr'00	-4
May'00	-4
Jun'00	-5
Jul'00	-5

Table 4.7 The monthly-mean Doppler-derived vertical velocity (mm s^{-1}) at 102 m in Inchmarnock Water during the period June 1999 to July 2000.

month, being strongest in August 1999 and weakest in March and May 2000. With a *VV* anomaly of –9 mm s^{-1} in August, and –4 mm s^{-1} in both March and May, the range of mean downward velocities would have been 1-10 mm s^{-1} and 1-5 mm s^{-1} respectively. These were generally associated with the deep, leading edge of the backscatter band during the dawn descent, suggesting that the fastest swimming at this time occurred by individuals that were just ahead of the bulk of the descending population.

The pre-dusk ascent thought to have been undertaken by *Calanus* (see above) was reflected by a marginal reduction in negative *VV* at 14 m in June/July 1999, and a greater reduction in August 1999 (Figure 4.16b). With a *VV* anomaly of –7 mm s^{-1} in June/July and –9 mm s^{-1} in August, the range of mean velocities of the ascending *Calanus* would have been 2-10 mm s^{-1} and 3-20 mm s^{-1}, respectively. There was only a hint of this ascent at 62 m (Figure 4.17b), indicating that individuals had probably not swum from much deeper than this. The arrival of the SSL around sunset, on the other hand, was evident at both 62 and 14 m by a reduction in negative *VV*. Similarly, the post-dusk midnight sinking inferred from the *Sv* data for the period June to December

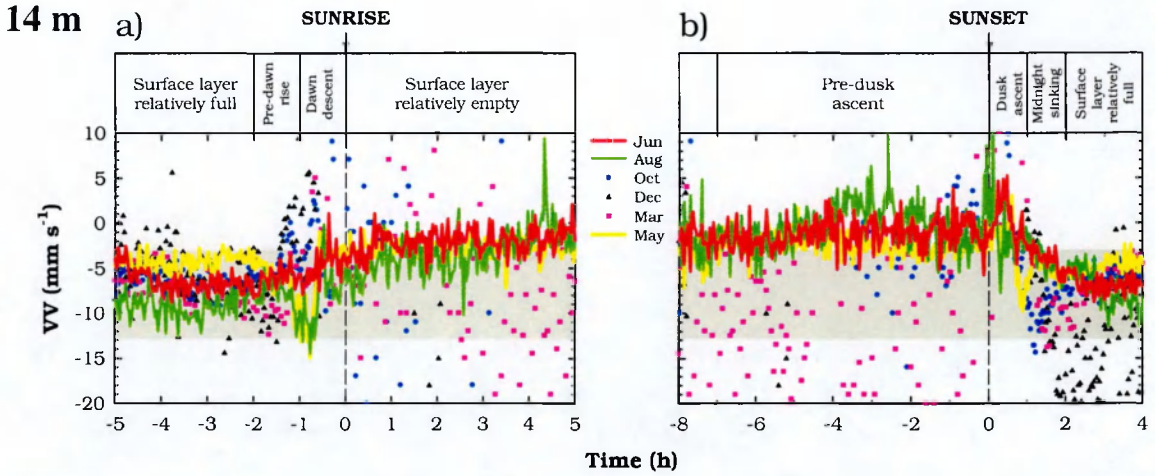

Figure 4.16 Changes in vertical velocity (*VV*, in mm s^{-1}) at 14 m depth around a) sunrise, and b) sunset, in Inchmarnock Water during the period June 1999 to May 2000. The shaded area represents the annual range of the vertical velocity 'anomaly' (see Table 4.7). The *VV* vectors in Oct'99, Dec'99 and Mar'00 were affected by the lack of a return signal at the surface at this time (see Discussion, section 4.4.2).

1999 (see above) was evident at both 62 and 14 m, in this case by an increase in negative *VV* (Figures 4.16b and 4.17b). These velocities appeared to be greatest in October, when both krill and *Calanus* were thought to be involved, and least in June/July, when only *Calanus* were thought to be involved. With a *VV* anomaly of –7 mm s^{-1} in June/July, –9 mm s^{-1} in August, –10 mm s^{-1} in October and –7 mm s^{-1} in December, the maximum velocities of the midnight-sinking *Calanus* would have been approximately 4 mm s^{-1}, 9 mm s^{-1}, 10 mm s^{-1} and 9 mm s^{-1}, respectively. As with the *Sv* data, the *VV* data in March and May 2000 suggested that midnight sinking was not taking place at these times.

Biometric measurements of Calanus

Sample size and data quality

Table 4.8 summarises the number of samples collected and the number of biometric measurements made during each sampling mission. These numbers are low in places for

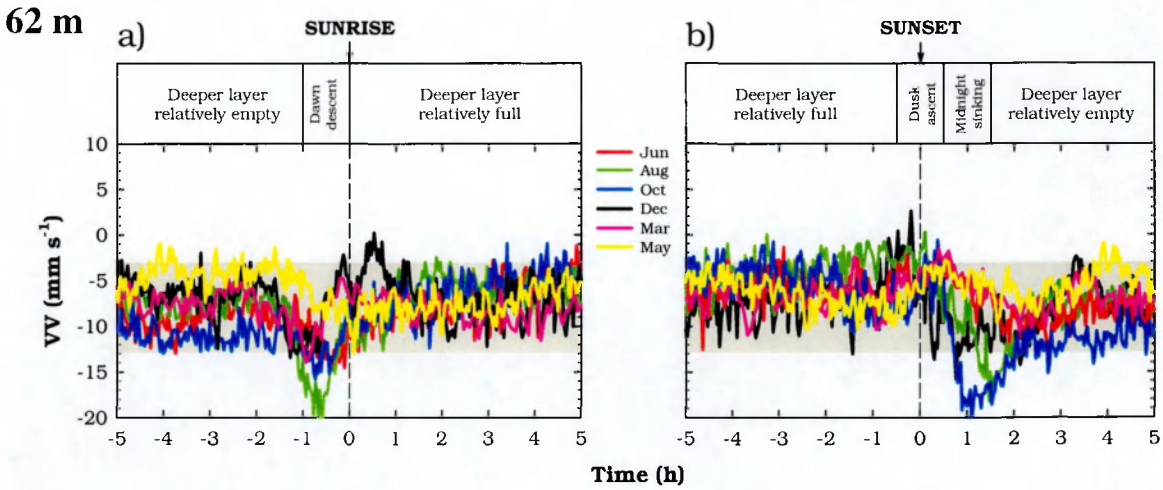

Figure 4.17 Changes in vertical velocity (*VV*, in mm s^{-1}) at 62 m depth around a) sunrise, and b) sunset, in Inchmarnock Water during the period June 1999 to May 2000. The shaded area represents the annual range of the vertical velocity 'anomaly' (see Table 4.7).

a variety of reasons. Firstly, gut fullness and dry weight measurements were not added to the sampling programme until March 2000. Secondly, only a proportion of the samples (~45 %) were measured for carbon and nitrogen weight, as it was intended to take a preliminary look at the results before making any further expenditure. However, the theft of a laptop computer, back-up disks and field-notes in June 2000 resulted in both the loss of some of the measurements already made, and an inability to cross-reference the samples pending analysis. While only a small amount of the data for *Calanus* was lost in this way, all of the data for *M. norvegica* and *T. raschii* were lost.

Table 4.9 shows the precision of the measuring instruments used, and the subsequent accuracy of the biometric data. By way of quality control, only those data values with an accuracy of ±5 % or less were deemed trustworthy enough for inclusion in further analyses (see Equation 2.2). Prosome length and carbon and nitrogen weight were measured with a relatively high degree of precision, such that all measurements lay well within the arbitrary accuracy threshold. Measurements of dry weight, however, varied considerably in their accuracy (±0.6-100 %), given that the equipment was relatively

			Individuals		Samples			
Ship visit	No. of net tows	*Calanus* stage	PL values	GF values	Total no.	Ind. per sample	DW values	C&N values
Jun'99	13	Females	62	0	32	1-3	0	32
		CV	90	0	33	1-4	0	29
		CIV	61	0	25	1-4	0	25
Aug'99	12	Females	105	0	37	1-4	0	12
		CV	121	0	42	1-4	0	13
		CIV	47	0	16	1-4	0	5
Oct'99	5	Females	48	0	20	1-3	0	9
		CV	74	0	27	1-3	0	0
		CIV	10	0	3	3-4	0	0
Dec'99	2	Females	25	0	22	1-2	0	8
		CV	96	0	68	1-2	0	20
		CIV	12	0	8	1-2	0	6
Mar'00	9	Females	76	76	41	1-3	40	37
		CV	87	87	27	1-4	27	8
		CIV	42	42	16	1-5	15	6
May'00	16	Females	171	171	46	1-6	46	0
			1127	**376**	**463**	**1-6**	**128**	**210**

Table 4.8 The number of *Calanus* stages CIV, CV and adult females collected from net tows in Inchmarnock Water and measured for prosome length (PL), gut fullness (GF), dry weight (DW) and carbon and nitrogen weight (C & N).

imprecise (±10 µg), and that the actual mass of the samples varied widely depending on the size of the individual being weighed and the number of individuals included per sample. With the ±5 % designated threshold and a measuring precision of ±10 µg, any measured dry weight <200 µg was therefore deemed too inaccurate for inclusion in further calculations (see Equation 2.2). Dry weight measurements discarded in this way included 12 of the 86 adult female samples, 7 of the 27 stage CV samples, and 12 of the 15 stage CIV samples.

Parameter	Precision	Data accuracy (%) Females	CV	CIV
Prosome length	± 0.04 mm	± 1.3 – 2.1	± 1.6 – 2.4	± 1.8 – 3.2
Dry weight	± 10 µg	± 0.6 – 11.1	± 1.4 – 33.3	± 1.8 – 100
Carbon & nitrogen weight	± 0.01 µg	± 0.1	± 0.1	± 0.1

Table 4.9 The precision and accuracy range of biometric measurements made on *Calanus* collected in Inchmarnock Water during the period June 1999 to May 2000.

Relationships between parameters: investigating the ecology of individuals

Figure 4.18 shows that the body weight (dry, carbon or nitrogen weight) of *Calanus* increased exponentially with increases in prosome length (i.e. $Y = aX^b$). The log transformation of the X and Y values therefore allowed this relationship to be described in linear terms (i.e. $\log Y = \log a + b \log X$). When all the data were combined, the log length/log weight relationships were relatively strong ($r^2 = 0.52$ to 0.80) and statistically significant (ANOVA: $P < 0.001$ in all cases). That is to say, changes in prosome length explained between 52 and 80 % of the changes in body weight. The bulk of the data deviated relatively little from a common regression line, implying that the length/weight 'body condition' of individuals from different depths, times of day, seasons and developmental stages was fundamentally similar. One notable exception, however, was the length/carbon relationship in December 1999 of most of the stage CV samples, and a smaller proportion of the stage CIV samples (Figure 4.18b). The obvious deviation of these data from the background pattern showed that certain individuals at this time had a better length/carbon 'body condition' than during any other sampling mission. From consideration of their depth of capture, it appeared as if those individuals with a better 'body condition' (i.e. more carbon per unit body length) were to be found closer to the surface both day and night.

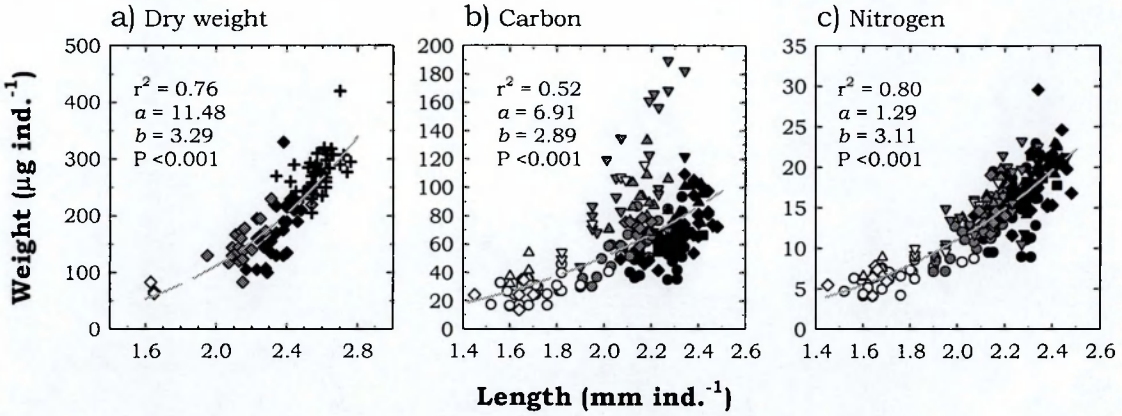

Figure 4.18 The relationship between the mean individual prosome length of *Calanus* (stages CIV, CV and adult females) in different samples (n = 1-6 individuals per sample) and the mean individual body weight, in terms of a) dry weight, b) carbon weight, and c) nitrogen weight. The lines of best fit ($Y = aX^b$) and their regression parameters are shown on each graph. Symbol colours define the developmental stage: CIV = white, CV = grey, adult females = black. Symbol shapes define the sampling date: Jun'99 = circles, Aug'99 = upward triangles, Oct'99 = squares, Dec'99 = downward triangles, Mar'00 = diamonds, May'00 = crosses.

Figure 4.19 shows that the body weight (dry, carbon or nitrogen weight) of size-normalised (2.18 mm-long) *Calanus* increased linearly with increases in gut fullness (i.e. $Y = a + bX$, $b > 0$). However, for adult females in May 2000, the apparent increase in dry weight (b = 0.128) was not significant at the α = 0.05 level (ANOVA: $F_{1,43}$ = 0.83, P = 0.367), meaning that gut contents in this case were probably not significant in terms of the overall mass of the individual. Conversely, for the combined developmental stages (CIV, CV and adult females) considered in March 2000, the observed increases in dry weight (b = 0.355) and nitrogen weight (b = 0.033) were significant at α = 0.05 (dry weight, ANOVA: $F_{1,50}$ = 6.1, P = 0.017; nitrogen, ANOVA: $F_{1,49}$ = 13.5, P <0.001). Furthermore, despite the fact that the increase in carbon weight (b = 0.097) was not significant at α = 0.05, it may well still have been real, given that there was only a 6.3 % probability that b = 0 (ANOVA: $F_{1,49}$ = 3.6, P = 0.063). Therefore, for *Calanus* in March at least, the weight of the gut contents appeared to be a statistically significant

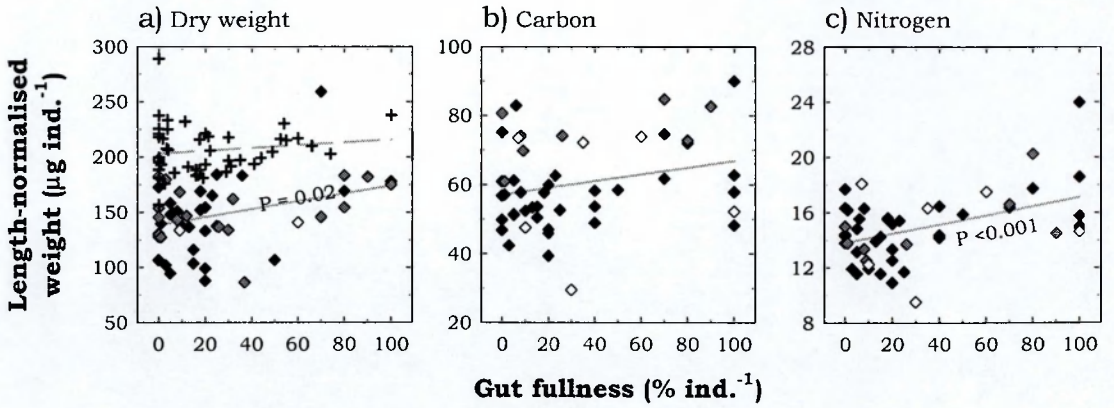

Figure 4.19 The relationship between the mean individual gut fullness of *Calanus* (stages CIV, CV and adult females combined) in different samples (n = 1-6 individuals per sample) and the mean length-normalised (2.18 mm) individual body weight, in terms of a) dry weight, b) carbon weight, and c) nitrogen weight. The lines of best fit ($Y = a + bX$) are shown on each graph (bold lines = Mar'00, broken line = May'00). The ANOVA: P value is appended to those lines for which b >0 (i.e. where ANOVA: P <0.05). See Figure 4.18 for an explanation of the symbols.

fraction of the overall weight (dry, carbon and nitrogen) of the individual. This suggests that defaecation might represent a relatively important avenue for carbon and nitrogen loss in this species at certain times. From the regression lines given in Figure 4.19, the weight of a size-normalised (2.18 mm-long) individual could be predicted when empty (GF = 0 %) and when full (GF = 100 %), and hence the weight of a gut-full of material in relation to the weight of the empty individual. These calculations revealed that a gut-full of material constituted 25.6 % of an individual's dry weight, 14.5 % of its carbon weight, and 24.1 % of its nitrogen weight.

Figure 4.20 shows that the carbon and nitrogen weight of *Calanus* increased linearly with increases in dry weight (i.e. $Y = a + bX$, b >0). Furthermore, these positive relationships were relatively strong (r^2 >0.5) and highly significant (ANOVA: P <0.001 in both cases), suggesting that dry weight might represent a proxy for both carbon and nitrogen weight. Therefore, where dry weight but not carbon or nitrogen weight measurements were made on a sample (some of the samples in March 2000, all of the

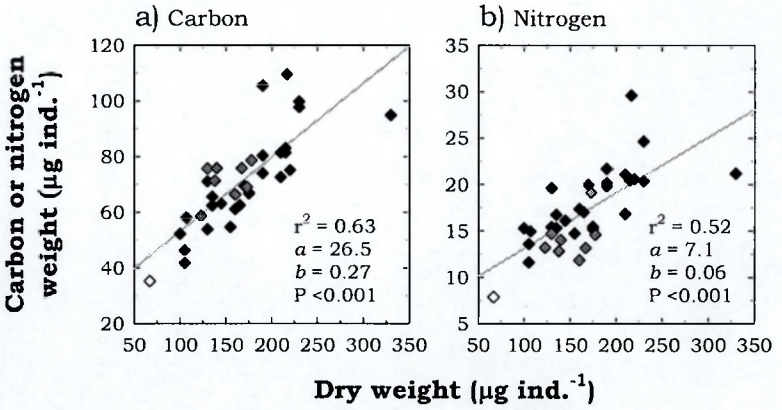

Figure 4.20 The relationship between the mean individual dry weight of *Calanus* in different samples (*n* = 1-6 individuals per sample) and the mean individual elemental composition, in terms of a) carbon weight, and b) nitrogen weight. The lines of best fit ($Y = a + bX$) and their regression parameters are shown on each graph. See Figure 4.18 for an explanation of the symbols.

samples in May 2000), these relationships were applied to predict the carbon and nitrogen weight, and therefore increase the sample size for more robust statistical analysis.

Figure 4.21 shows that the nitrogen weight of *Calanus* increased linearly with increases in carbon weight (i.e. $Y = a + bX$, $b > 0$). Also apparent, however, is the large amount of seasonal, intra- and inter-specific variability in the C:N ratio (atoms). This suggests a large amount of individual variability in the relative proportions of lipids and proteins. C:N in 80 % of the samples ranged between 3.8 and 6.5 (relatively high protein content), with representatives from each sampling month and each developmental stage, while the C:N of the remaining 20 % ranged between 6.5 and 10.1 (relatively high lipid content). Of this remaining 20 %, roughly half was made up of stage CV samples collected in December 1999, while a quarter was made up of stage CV samples collected in August 1999. For the December samples, a consideration of the depth of capture suggested that those individuals with a higher C:N (i.e. more lipid) were to be found closer to the surface, and that it was these individuals which were

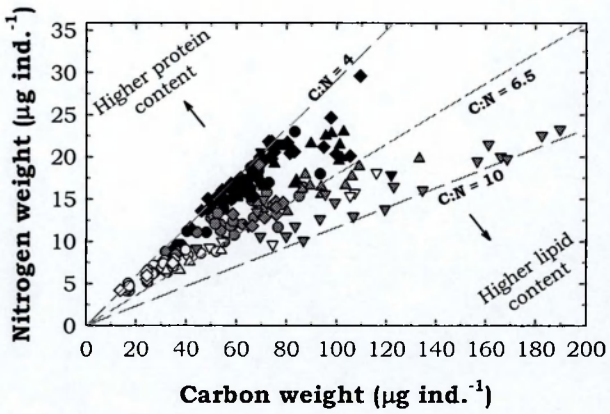

Figure 4.21 The relationship between the mean individual carbon weight of *Calanus* in different samples (n = 1-6 individuals per sample) and the mean individual nitrogen weight. Lines show the C:N (atoms) lines of equivalence. See Figure 4.18 for an explanation of the symbols.

undertaking DVM. Overall, stage CIV and adult female individuals exhibited C:N ratios closer to the lower end of the range (mean±1SD: females = 4.6±0.7, stage CIV = 5.6±1.2), while stage CV individuals exhibited C:N ratios closer to the higher end of the range (mean±1SD = 6.6±1.6).

Variability in the data

Table 4.10 shows the mean, standard deviation (SD) and coefficient of variation (V) of the various biometric measurements made on *Calanus* collected in Inchmarnock Water during the period June 1999 to May 2000. The data were combined from each sampling date, but considered separately for stages CIV, CV and adult females. The size-normalisation of the dry-, carbon- and nitrogen-weight measurements to a mean prosome length of 2.18 mm (see section 2.3.6) strongly reduced V in all but one instance, demonstrating that differences in the size of individuals between samples represent a significant source of variability in the data, and highlighting the importance of performing the size-normalisation procedure. For a number of samples collected in

		Mean±1SD (*V* %)		
Parameter	Analysis	Females	CV	CIV
Prosome length (mm)	Raw measure	2.40±0.18 (7.39)	2.12±0.14 (6.52)	1.69±0.17 (10.06)
Dry weight (µg)	Raw measure	237.9±63.8 (26.8)	151.3±36.0 (23.8)	71.1±10.7 (15.1)
	L-norm.	181.5±41.5 (22.9)	153.0±26.4 (17.2)	152.7±27.0 (17.7)
Carbon weight (µg)	Raw measure	68.2±17.5 (25.6)	81.8±35.6 (43.6)	37.8±23.6 (62.5)
	L-norm.	58.9±12.6 (21.4)	86.2±31.7 (36.8)	65.5±21.6 (33.0)
Nitrogen weight (µg)	Raw measure	17.3±3.6 (20.7)	14.2±3.5 (24.5)	7.5±3.0 (39.6)
	L-norm.	14.7±2.5 (17.3)	15.2±2.6 (17.3)	14.3±2.7 (19.0)

Table 4.10 The mean, standard deviation (SD) and coefficient of variation (*V*) of the various biometric measurements made on *Calanus* collected in Inchmarnock Water during the period June 1999 to May 2000. The data are combined from all sampling months, and considered separately for each developmental stage (stages CIV, CV and adult females). 'L-norm' refers to those measurements that were standardised to a mean prosome length of 2.18 mm using ANCOVA (see section 2.3.6).

March 2000, concurrent measurements of prosome length and dry, carbon and nitrogen weight were made. This allowed an assessment to be made as to which measure of body size was the most effective to use for the size-normalisation procedure. When carbon weight was normalised to a mean dry weight of 1.5 mg, *V* was less than when normalised to a mean prosome length of 2.18 mm (*V* = 15.8 % vs. 21.1 %). For nitrogen weight, however, the reverse was true (*V* = 18.8 % vs. 16.6 %), suggesting that neither measure of size was more effective than the other, at least in this instance. Data were therefore normalised to a mean length of 2.18 mm throughout.

The size-structure of the population from prosome length measurements

Figure 4.22 shows that stage-specific individuals of *Calanus* (stages CIV, CV and adult females) exhibited a fairly wide range of lengths at any one time. Combined with the fact that the size-distributions were non-normal in most cases (Kolmogorov-Smirnov: P

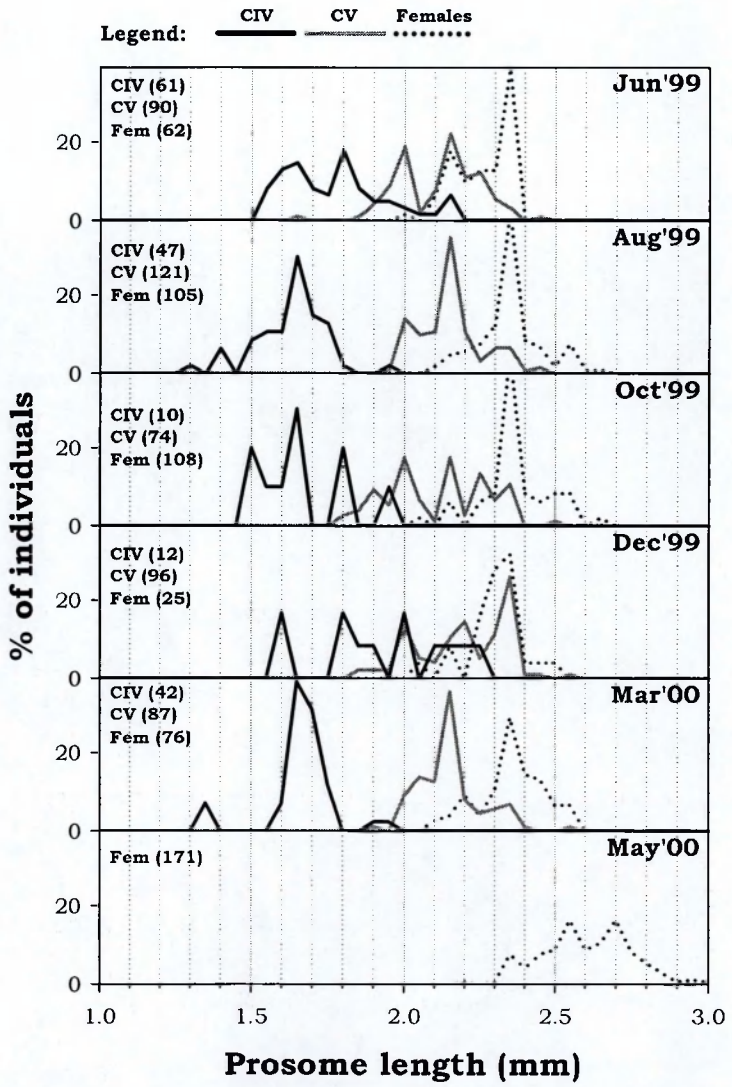

Figure 4.22 The prosome-length frequency (0.05 mm length classes) of *Calanus* from Inchmarnock Water during the period June 1999 to May 2000, showing the distribution of stages CIV, CV and adult females. The number of individuals is shown in brackets on each graph.

≤0.01), this implied that the stage-specific stocks contained a heterogeneous mixture of individuals in terms of length. In Table 4.10, it was shown that the variability (V) in the length of individuals at any one time ranged from 6.5 to 10.1 %. While this was low compared to the dry-, carbon- and nitrogen-weight data, it was of a sufficient magnitude to suggest that one or more factors were acting to create variability within the size-structure of the population. Despite the potential for this variability to mask any potential temporal differences in prosome length, significant changes were indeed

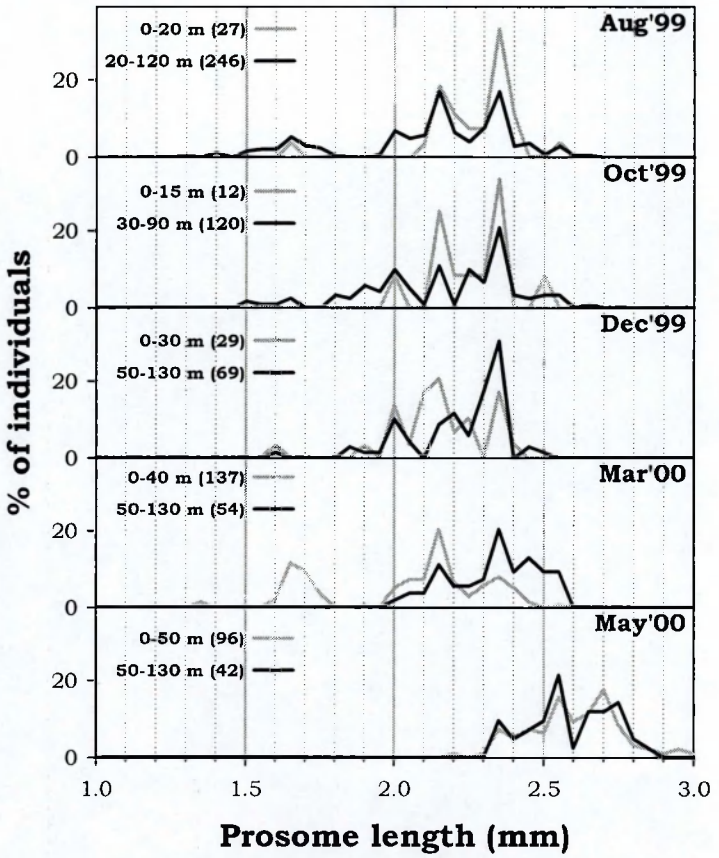

Figure 4.23 The prosome-length frequency (0.05 mm length classes) of *Calanus* from Inchmarnock Water during the period August 1999 to May 2000, showing the distribution of combined stages (CIV, CV and adult females) in different depth strata where concurrent samples were available. The number of individuals is shown in brackets on each graph.

apparent in the median length of each developmental stage over the course of the year (Kruskal-Wallis: $P < 0.001$ in each case). In general, adult females were longest in May 2000 and shortest in June/July 1999, while stage CV and CIV were longest in December 1999 and shortest in October 1999 and August 1999, respectively.

Figure 4.23 shows that, in those months where concurrent samples at different depths were available, there was little evidence for any change in the length distribution of individuals with depth (stages CIV, CV and adult females inclusive). That is to say, these data did not suggest that smaller individuals within the population were to be found closer to the surface, or *vice versa*. There were a few instances in which time- or

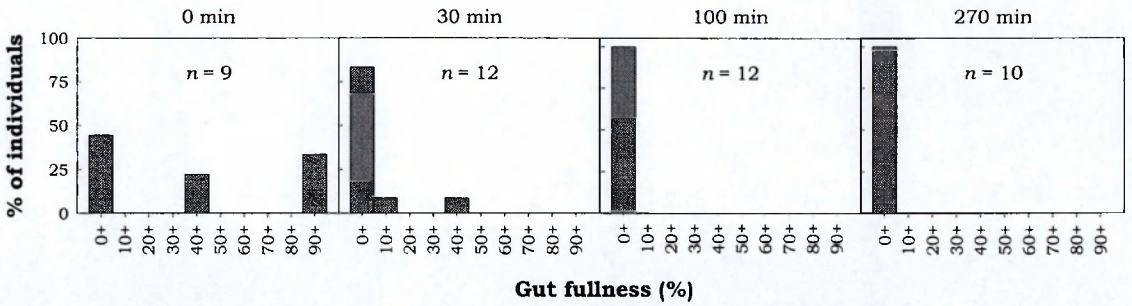

Figure 4.24 The gut-fullness distribution of *Calanus* adult females collected at dawn in Inchmarnock Water in March 2000 and maintained for varying lengths of time in the absence of food (incubation in 0.45 μm-filtered seawater at 11 °C).

depth-related differences in stage-specific length distribution may have been apparent (Kruskal-Wallis: $P \leq 0.05$; data not shown), but the magnitude of these differences was generally small (<5 % difference in length).

Diel patterns of feeding from gut fullness measurements

Temporal changes in the gut fullness of starved *Calanus* adult females were investigated via a shipboard time-course experiment conducted in March 2000 (incubation in 0.45 μm-filtered seawater at 11 °C). Figure 4.24 shows that the gut-fullness distribution of experimental individuals changed noticeably over the 4-5 h incubation period. These differences were found to be significant (Kruskal-Wallis: $H_3 = 12.63$, $P = 0.006$). The observation that that no individuals were found to be more than 50 % full after 30 min of starvation, and none more than 5 % full after 100 min, implies that complete evacuation of the guts would have taken somewhere between 30 and 100 min under these experimental conditions.

Table 4.11 shows that, in March 2000, the range of gut-fullness values of field-sampled *Calanus* was similar for each stage (CIV, CV and adult females) at any given depth stratum and time of day. This suggested that they had similar diel feeding patterns. These stages were therefore combined in order to increase sample numbers for

		Females		CV		CIV	
Time (h)	Depth (m)	GF (%)	*n* (ind.)	GF (%)	*n* (ind.)	GF (%)	*n* (ind.)
06:00	0-25	20-100	10	0-100	16	40-100	4
07:00	0-120	0-100	9	0-90	2	0	3
09:30	0-15	40	1	5	1	30	1
09:30	50-75	5-30	4	0	4	-	0
09:30	100-130	5	3	0	2	-	0
12:00	0-15	-	0	0-100	6	0-100	11
12:00	50-75	0-20	8	0-5	5	-	0
15:00	0-15	0-5	4	0	3	0	2
15:00	50-75	0-100	24	0	4	-	0
18:00	0-40	0-50	6	0-100	17	0-70	12
19:00	0-15	0-100	7	0-100	27	0-30	9

Table 4.11 The gut fullness (%) range and number of individuals measured (*n*) for *Calanus* stages CIV, CV and adult females collected at different times and depths in Inchmarnock Water in March 2000.

more robust statistical analysis. Only adult females were sampled in May 2000, so such a combination was not necessary. Figure 4.25 shows the gut-fullness distribution of individuals collected from the field at different times of the day and night in March (combined stages) and May (adult females only). The strong similarities between each month suggested that the patterns of feeding were similar. In both months, there were significant differences between the median gut fullness values at each time-point (March, Kruskal-Wallis: $H_{10} = 71.317$, $P < 0.001$; May, Kruskal-Wallis: $H_{14} = 65.057$, $P < 0.001$), suggesting that levels of feeding varied over the diel cycle.

Throughout the night, the surface-caught samples had a bimodal distribution. The presence of fuller individuals indicated that feeding had been taking place, while the presence of emptier individuals indicated one of three possibilities: (1) the guts were being filled and emptied more than once during the night, (2) a proportion of the population was not feeding, yet remaining at the surface at night, or (3) individuals had just arrived in the surface layer from below, where they had not been feeding. As light

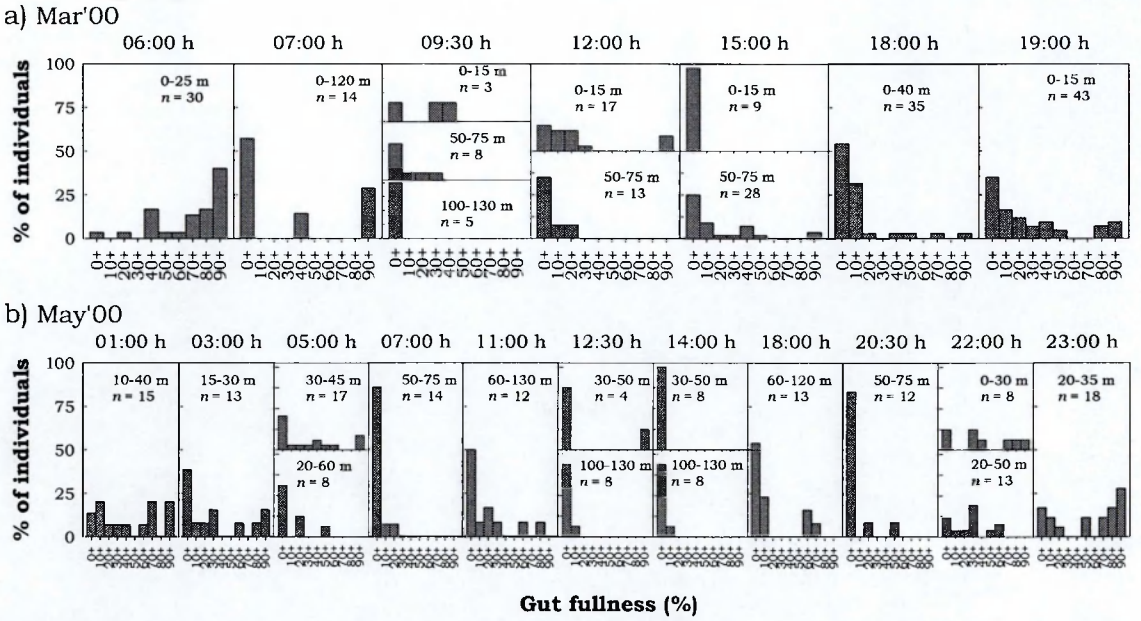

Figure 4.25 The gut-fullness distribution of *Calanus* collected at different times of the day and night in Inchmarnock Water in a) March 2000 (stages CIV, CV and adult females combined), and b) May 2000 (adult females only).

levels increased through the dawn and early morning, a larger proportion of emptier individuals were found. Indeed, the continued predominance of emptier animals throughout the day strongly suggested a cessation or reduction in feeding during daylight hours. However, the presence of some individuals up to 100 % full during the day in both months was indicative that at least some of the population had been feeding recently. Furthermore, the fuller individuals were generally caught closer to the surface. As light levels decreased towards dusk, a return to the bimodal nighttime gut-fullness distribution was observed. This was indicative of a switch from the daytime mode of reduced feeding, and a return to the nighttime levels of increased feeding.

Diel changes in carbon and nitrogen weight

Temporal changes in the carbon and nitrogen weight of starved *Calanus* adult females were investigated via two shipboard time-course experiments conducted in August 1999

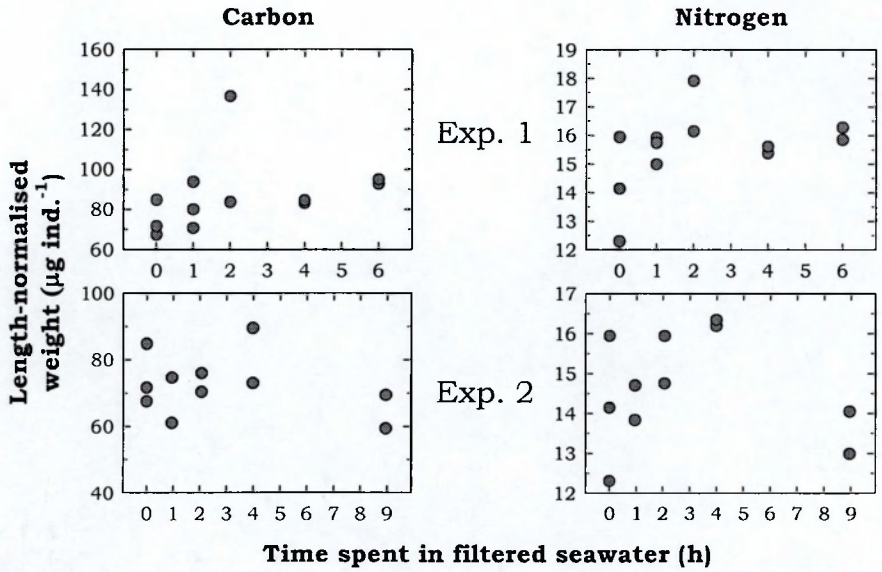

Figure 4.26 The carbon and nitrogen weight of size-normalised (2.18 mm-long) *Calanus* adult females collected at dawn in Inchmarnock Water in August 1999 and maintained for varying lengths of time in the absence of food (incubation in 0.45 µm-filtered seawater at 9 °C). All values were measured directly (as opposed to being derived from dry weight).

(incubation in 0.45 µm-filtered surface seawater at 9 °C). No carbon or nitrogen weights are available for the experiment conducted in March 2000 due to the loss of data as previously explained. Figure 4.26 shows that there was no systematic pattern of change in either the carbon or the nitrogen weight of size-normalised (2.18 mm-long) individuals over time in either of the August experiments. Furthermore, ANOVA confirmed that there were no significant differences between time-points in either experiment (ANOVA: $P > 0.05$ in all cases). Variability (V) in both carbon and nitrogen weight was actually relatively low at each time-point, ranging from 0.5 to 34 % (mean = 9 %). All carbon and nitrogen weights were directly measured, rather than derived from dry weight.

Figure 4.27 shows the carbon and nitrogen weights of size-normalised (2.18 mm-long) individual *Calanus* (stages CIV, CV and adult females combined) collected from the field at various times of the day and night in June/July, August and December 1999,

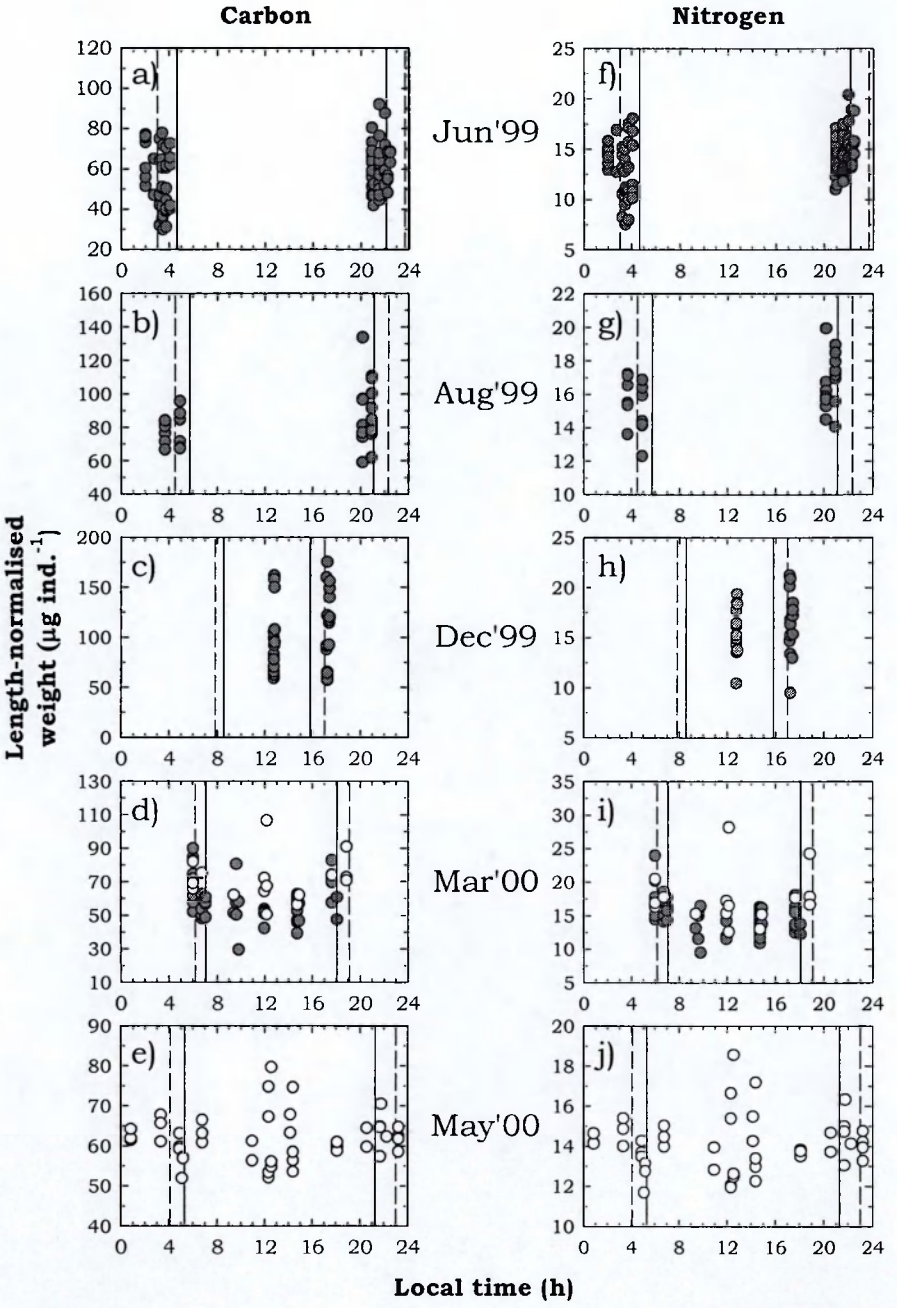

Figure 4.27 Diel differences in the carbon and nitrogen weight of size-normalised (2.18 mm-long) *Calanus* (stages CIV, CV and adult females combined) collected in Inchmarnock Water during the period June 1999 to May 2000. Broken lines represent the times of first and last light. Solid lines represent the times of sunrise and sunset. Filled symbols represent those values measured directly. Empty symbols represent those values derived from dry weight (see Figure 4.20 for the regression parameters used).

and March and May 2000. No data are available for October 1999 due to the loss of data as previously explained. No obvious diel patterns were apparent in any month, while a dawn-dusk comparison was not available for samples collected in December due to

marginal weather conditions. In June/July, there were significant differences between both the carbon- and nitrogen-weight means at each time-point (carbon, ANOVA: $F_{2,83}$= 3.533, P = 0.034; nitrogen, ANOVA: $F_{2,83}$= 7.507, P = 0.001). While a Tukey's test failed to pick up which groups differed from which in terms of carbon, with nitrogen this difference was primarily due to individuals at dusk containing more (2.3 μg N) than those at dawn (Tukey's: P <0.001). The pattern in August was similar, although no significant differences were found in carbon weight (ANOVA: $F_{2,27}$= 1.659, P = 0.209), while a significant change was seen in nitrogen weight (ANOVA: $F_{2,27}$= 3.768, P = 0.036). Again, a Tukey's test showed that individuals at dusk contained more nitrogen (1.9 μg N) than those at dawn (Tukey's: P = 0.032). Significant diel changes were also found in March (carbon, ANOVA: $F_{10,56}$=3.733, P <0.001; nitrogen, ANOVA: $F_{10,56}$= 2.924, P = 0.005). However, no systematic diel pattern was apparent for either carbon or nitrogen, and a Tukey's test did not pick up which groups differed from which. No significant diel changes were found in May (carbon, ANOVA: $F_{13,31}$=0.496, P = 0.910; nitrogen, ANOVA: $F_{13,31}$=0.503, P = 0.905). None of these findings are consistent with the idea that individuals were feeding more at night than during the day.

4.3.4 Statistical assessment of the success of ZOOFLUX in the Clyde Sea

Table 4.12 shows the statistical success of the first field-application of ZOOFLUX, based on *Calanus* (stages CIV, CV and adult females) collected in Inchmarnock Water in June/July and August 1999, and March and May 2000 (see section 2.3.8 for an explanation of the calculations used).

The number of samples available for the dawn-dusk comparisons (*n*, expressed as the mean of the number collected at both dawn and dusk) were low in all cases (6 to 36 samples), while the variability in the carbon and nitrogen measurements (*V*, expressed

Date	Element	n	δ %	V %	ANOVA: P	β %	n_{min}	δ_{min} %
Jun'99	Carbon	36	-15	23	0.011	n/a	49	18
	Nitrogen	36	-19	20	<0.001	n/a	23	15
Aug'99	Carbon	12	-14	18	0.192	87	61	32
	Nitrogen	12	-13	10	0.018	n/a	17	15
Mar'00	Carbon	13	2	15	0.738	95	1300	20
	Nitrogen	13	8	18	0.218	89	93	23
May'00	Carbon	6	-10	7	0.045	n/a	13	16
	Nitrogen	6	-11	7	0.044	n/a	13	17

Table 4.12 The statistical 'success' of ZOOFLUX in Inchmarnock Water (see section 2.3.8), based on the carbon and nitrogen weight of size-normalised (2.18 mm-long) *Calanus* (stages CIV, CV and adult females combined) collected at dawn and dusk. n = the mean of the number of samples collected at dawn and at dusk. δ = the dawn-dusk difference in carbon or nitrogen weight. V = the mean coefficient of variation of the carbon or nitrogen data from both dawn and dusk. β = the probability of committing a Type II error (when ANOVA: P >0.05). n_{min} = the minimum number of samples required in order to detect a significant δ (ANOVA: P ≤0.05). δ_{min} = the minimum detectable dawn-dusk difference.

as the mean of the coefficients of variation at both dawn and dusk) was relatively high (7 to 23 %). In six out of eight comparisons, the dawn-dusk difference (δ, expressed as a percentage change) was actually seen to be negative (i.e. carbon and/or nitrogen weight actually seemed to have increased during the day). In three out of eight comparisons, δ was found to be non-significant (ANOVA: P >0.05), and the probability that a Type II error had been made ($\beta \times 100$) was high in each case (>85 %). The variability (V) in the data was relatively high in each of these instances (15 to 18 %), and sample numbers were relatively low (10 to 13 samples).

Overall, the minimum number of samples that would have been required (n_{min}, expressed as the mean) in order to conclude that the observed dawn-dusk differences were significant (ANOVA: P ≤0.05) ranged widely (13 to 1300 samples). In only one instance was this number less than the number of samples actually collected (June/July;

nitrogen), and in three out of eight cases the number required was not realistically obtainable (>50 samples). The magnitude of n_{min} related less to either the sample size (n) or the variability in the data (when expressed as V), and more to the particular source of the variability. Specifically, the higher the error MS in relation to the groups MS (see section 2.3.8), the higher the value of n_{min}, and *vice versa* (recall that Equation 2.11 employs only error MS as the variability term). That is to say, the variability within the data at either dawn or dusk was more important in deciding the minimum number of samples required than the variability between the data at each of these time-points. Finally, the minimum dawn-dusk difference that would have been detectable (δ_{min}) in each case ranged from 15 to 32 %.

4.4 Discussion

This study set out to investigate the role of zooplankton diel vertical migrants in the removal of carbon and nitrogen from the surface mixed layer of the ocean to the depths. Inchmarnock Water, in the Clyde Sea Area, provided a convenient site for such a study. Net tows revealed that the most likely mesozooplankton contributors to an active flux at this site were *Calanus* and krill (Figures 4.8 to 4.11), and acoustic measurements helped to elucidate the timing and amplitude of their vertical movements with a high degree of resolution (Figures 4.12 to 4.17). These methods showed that the DVM behaviour of *Calanus* was complex and variable, an observation that appears to be common in the literature (see section 4.1.4). Furthermore, biometric measurements revealed there to be a great deal of variability in the physiology of individuals (Figures 4.18 to 4.27). This information, along with measurements of the physical environment (Figures 4.5 and 4.6) and assessments of the potential food sources available to migrants (Figure 4.7 and Table 4.5), now allows a number of active-flux related questions to be posed: (1) Was

the physical environment conducive to an active flux?; (2) How and why were migrants behaving as they did, and what were the consequences of this to the ecosystem?; (3) Was an active export flux of carbon or nitrogen actually occurring at this site, and, if so, could it be quantified accurately using the present dataset? These questions are addressed in turn below.

4.4.1 The physical environment

As Nicholls (1933) wrote, "The Clyde Sea Area is peculiarly suitable for work on vertical distribution of such an animal as *Calanus*, owing to the presence of large bodies of water practically cut off from the rest of the sea, and subject to only small tidal movements". How suitable, though, is this region for work on the active flux caused by zooplankton diel vertical migration?

Bathymetry

Water depth at the Inchmarnock Water study site was ~160 m. While this was certainly deep enough for stratification and interzonal DVM to have been features of the environment at various times of year, in terms of the maximum potential amplitude of the significant migrant species, this was relatively shallow. For example, *Calanus* is capable of migrating as deep as 1500 m (e.g. Hirche, 1991) in regions with sufficient water depth. Similarly, *M. norvegica* is capable of migrating as deep as 1500 m, and *T. raschii* as deep as 200 m or more (see Mauchline, 1980, for references). In this way, the presence of the seafloor at 160 m is likely to play a constraining role in the vertical distribution of each of these species. For both *M. norvegica* and *T. raschii*, which are known to associate with the benthos and feed on resuspended detrital material (see e.g. Mauchline, 1980; Youngbluth *et al.*, 1989), there is therefore the potential for

significant feeding during the day. This means that an active upward flux of benthic material may occur when animals that have been feeding at depth during the day ascend to the surface at dusk. If this outweighs the amount of material ingested in the surface layers during the night and carried to depth at dawn, then the behaviour of these species will in fact be responsible for returning previously sequestered material to the euphotic zone. A further consequence of this behaviour is that daytime populations closely associated with the benthos will prove difficult to sample effectively using conventional net tows. Similarly for *Calanus*, despite the possibility that it will avoid residing too close to the benthos (where both krill predators and parasites may well be abundant), the relative proximity to the seafloor is likely to result in above-average levels of resuspended detritus at daytime-residence depths that would enable daytime detritivory and a further potential for upward flux to occur. Pearre (2003) provided a number of examples from the literature of upward fluxes by zooplankton in both marine and freshwater environments. Perhaps the most relevant of these to the present study are those of Youngbluth *et al.* (1989), who suggested that *M. norvegica* may have been returning ingested benthic material back into the mixed layer in the Gulf of Maine, and Saiz & Alcaraz (1990), who suggested that the copepod *Centropages typicus* may have been returning nutrients ingested in the deep chlorophyll layer back into the surface waters in the western Mediterranean.

Hydrography

For the Clyde Sea Area in general, a pycnocline is evident only during the summer months (June to October). This means that, for any material that may be exported from the surface waters, it is only during this period that it is likely to remain trapped at depth (based on the understanding that the pycnocline is the primary barrier to upward

mixing). Longhurst *et al.* (1990) touched on this in their discussion of the active respiratory DIC flux: "where such a barrier exists only seasonally the same transport may occur, though without the same significance, because winter mixing will subsequently redistribute the respired carbon". In the Clyde Sea, the relative shallowness of the water column is likely to have a reductive effect on the deep residence time of sequestered material. At only 160 m deep, the potential exists for complete mixing and convective overturn of deep waters in the winter, as was indeed recorded in December 1999 and March 2000 (Figure 4.5). As Edwards *et al.* (1986) estimated, the waters of the Arran Deep may be completely renewed approximately once a month during periods of complete winter mixing. With a residence time of ~2 months for the waters of the Outer Firth, it is possible that, no matter how much material is being exported to depth in Inchmarnock Water during the year, anything that does not become consolidated within the sediments could well be recirculated into the surface waters of the North Channel within 6 months or less. This suggests that, while Inchmarnock Water might still be considered a useful site for studies of zooplankton ecology, it is not a site at which a long-term sequestration of biogeochemically important elements is likely to be occurring.

4.4.2 Mesozooplankton behaviour and ecosystem consequences

Aside from purely scientific interest, if we can understand the way in which the mesozooplankton community was behaving and why, then we can understand better the processes controlling whether or not an active flux would have been occurring. The net-catch data from the present study (Table 4.5) showed that the Inchmarnock Water mesozooplankton community was fairly diverse and temporally variable in terms of both species composition and numerical abundance. Stratified day/night tows (Figures

4.8 to 4.11) indicated that the most significant vertical migrators in terms of amplitude were *Calanus* (stages CIV, CV and adult females) and krill (*M. norvegica* and *T. raschii*), and that interzonal DVM was likely to have been occurring during the stratified summer months. The acoustic data (Figures 4.12 to 4.17) significantly enhanced this information. Validation against the net catches showed that different taxa were responsible for different acoustic patterns, while the continuous nature of the data revealed patterns of DVM behaviour on time and space scales not detectable with discrete net tows.

Interpreting the net-catch and acoustic data

Before attempting to reconstruct the true nature of DVM at this site, one must first consider what exactly the net-catch and acoustic data can say. First of all, the spatial and temporal resolution of the net tows reflected on our ability to describe the precise amplitude and timing of DVM. In his often-cited assessment of such issues, Pearre (1979a) wrote "simple counts of organisms as functions of time and depth, or sonic scattering records, can yield only minimal estimates of range or velocity of vertical migrants". He therefore suggested "supplemental sources of information" from stratified samples, which included the analysis of changes in size/age and/or gut fullness spectra at different depths. In his most recent review, Pearre (2003) discussed more such useful "tracers" for individual movements. In addition to observing gut fullness, movements may be deduced from the presence of specific food items in the gut in relation to the depth of capture and the known vertical distribution of these items in the water column. Furthermore, the potential for an individual to migrate may be deduced from physiological factors such as body protein and carbon weight, and the activity of the electron transport system (ETS), while evidence that an individual has migrated may be

inferred from parameters such as amylase activity, body phosphorus weight and radioisotope tracers such as ^{131}I, ^{33}P and ^{14}C. While the biometric parameters measured in the present study were potentially useful tracers for deciphering the vertical movements of individual *Calanus*, it is unfortunate that the sampling schedule did not often yield concurrent samples from different depth strata to allow such an analysis to be undertaken.

A relatively new tool that promises to circumvent some of these net-associated issues is the ADCP. However, while this instrument is capable of providing biological information on superior time and space scales to net tows, data interpretation is subject to a number of often-complex considerations. We shall turn first to the interpretation of the backscatter (*Sv*) data shown in Figure 4.12. According to the modelling considerations of Francis *et al.* (1999a), the target strength (*TS*) of a 2.4 mm (presumably prosome length) individual *Calanus finmarchicus* at 300 kHz should be between –105 and –115 dB, depending on its orientation relative to the sound source. Similarly, Ashjian *et al.* (1998) calculated a theoretical *TS* of –100 dB. These values are lower than the minimum *Sv* measured in this study (–95.31 dB), suggesting that individual *Calanus* had not been detected. The individual *TS* of krill such as *M. norvegica* and *T. raschii*, on the other hand, is greater than that of *Calanus*, and will be expected to range from –70 to –100 dB at 300 kHz (e.g. Francis *et al.*, 1999b; Stanton & Chu, 2000; de Robertis, 2001). One might therefore suggest that it was these species that had contributed to the backscatter measured during this study. However, two other factors must also be considered. Firstly, absolute backscatter (*Sv*, in dB) is a function not only of *TS*, but also of the numerical concentration of scatterers (*N*) according to the equation given by MacLennan & Simmonds (1992):

$$Sv = 10\log N + TS$$

Equation 4.2

Secondly, if these scatterers are present in a region in which there is background backscatter (from e.g. microzooplankton, suspended detritus, sediment etc.), then this background will also be included in the *Sv* value registered by the instrument. In other words, in a region containing, for example, *Calanus* and/or krill, the measured *Sv* will be a combination of the backscatter from these animals plus the background signal. In this study, the background signal ranged from –80 to –95 dB in regions where it was known, from net tows, that relatively few of the larger backscattering particles were present.

Theoretically, in a region with no background backscatter, an observed *Sv* of –80 dB, for example, would equate to 100-3000 *Calanus* m^{-3}, or 1-100 krill m^{-3} (from Equation 4.2), depending on the exact size and orientation of individuals and assuming monospecific aggregations. At –70 dB, these concentrations would have to be greater: 1000-40000 *Calanus* m^{-3}, or 1-1000 krill m^{-3}. The inclusion of background backscatter within the measured *Sv* value would have the effect of reducing these concentrations, thereby enhancing the apparent sensitivity of the ADCP. From these considerations, and using the *TS* values reported above for *Calanus* and krill, the *Sv* data from the present study can be divided into three categories and interpreted in the following way:

1. **High *Sv* (–47 to –70 dB).** Also referred to here as the sound scattering layer (SSL), backscatter within this range could not have been due exclusively to *Calanus*, unless present in concentrations >1000 ind. m^{-3} (this was not likely from the net-catch data). Not even the addition of background backscatter at –80 dB could reduce this concentration to a realistic level. Therefore, *M. norvegica* and/or *T. raschii* must

have been present, either exclusively, or in conjunction with *Calanus*. Furthermore, the net-tow derived krill concentrations were generally <1 ind. m^{-3} (see Figure 4.9). At these low concentrations, bearing in mind that they may well have been underestimates due to net avoidance, monospecific assemblages could only have been detected if they contained individuals at the higher end of the *TS* range (i.e. –70 dB). For *Sv* values > –70 dB, it is more likely that the higher backscatter signal was due to the added presence of either background backscatter and/or high concentrations of *Calanus*.

2. **Medium *Sv* (–70 to –80 dB).** Could have been due to dense monospecific aggregations of *Calanus* (100-1000+ ind. m^{-3}), although net-tow derived concentrations averaged <20 ind. m^{-3} and never exceeded 125 ind. m^{-3} (Figure 4.8). However, including a high background backscatter of, say, –80 dB, would mean that this observed backscatter could have resulted from concentrations in the order of 40-400 ind. m^{-3}. It is likely, though, that *M. norvegica* and/or *T. raschii* would also have been present in this *Sv* range. Again, however, the individuals present would have to have been at the higher end of the *TS* range, given the low net-tow derived concentrations (e.g. at a monospecific concentration of 0.5 ind. m^{-3}, the minimum detectable *TS* would be –75 dB). This *Sv* category is therefore likely to have consisted of krill, but with *Sv* values, especially towards the higher (–70 dB) end of the scale, enhanced by either background backscatter and/or higher concentrations of *Calanus*.
3. **Low Sv (–80 to –95 dB).** Could have been due to monospecific aggregations of *Calanus* with concentration ranges of 3-100 ind. m^{-3} (when *TS* = –100 dB) or 100-3000 ind. m^{-3} (when *TS* = –115 dB). Again, these concentration ranges would have been lower with the addition of a background signal. Since this *Sv* range could not

have been due to concentrations of krill in excess of 0.1 or 100 ind. m^{-3} (with $TS = -70$ and -100 dB respectively), it is more likely that values within this range were typically caused by *Calanus* alone and/or the background backscatter.

Turning next to the vertical velocity (*VV*) data shown in Figure 4.13, the observation of consistently negative (i.e. downward) vertical velocities may have been an artifact caused by one or more of the following: (1) instrumental bias; (2) the positioning of the mooring in an area of downwelling; (3) the nature and orientation of the individual backscattering particles. The indications that this downward 'anomaly' changed seasonally (Table 4.7) strongly implied that there was an environmental rather than an instrumental causation. In Tarling *et al.* (2002), where the same dataset was considered, the *VV* data were plotted relative to this anomaly (albeit calculated in a different way to that in Table 4.7) so that the plots were somewhat easier to interpret, i.e. negative *VV* values represented downward movement of biomass, and *vice versa*. However, the decision on how to quantify this anomaly at any one time is somewhat arbitrary, and the 'raw data' are purposely shown here in order to avoid this tricky issue. While it seems likely that the primary cause of this anomaly was downwelling (since the magnitude of downwelling is likely to have changed seasonally in a way similar to that of the observed anomaly), one must also consider the nature and orientation of the backscattering particles. For example, *Calanus*, which has been shown (above) to be a likely contributor to the acoustic backscatter at 300 kHz, is likely to perform several different patterns of swimming behaviour (see Figure 83 in Mauchline, 1998, p.404). It is possible that individuals with outstretched antennae, for example, might provide a stronger backscatter return than those with their antennae by their sides. If individuals were to move upwards with their antennae down, and downwards with their antennae

outstretched (e.g. "hop and sink", or "cruise and sink" behaviour), then we might expect to find a downwards bias in the *VV* data. It is also worth mentioning that the *VV* values are likely to be an underestimate of the true swimming speeds of individuals. Part of the reason for this was explained by Pleuddemann & Pinkel (1989): "Since the migrating scatterers are typically only a small proportion of the scattering field, the quasi-stationary background makes a significant contribution to the total backscattered intensity and tends to "bias" the Doppler velocities to values smaller than the true migration rates". The other reason is that the *VV* values represent time-averaged data for all of the particles within a given volume of water. Unless these were all moving in the same direction and at the same speed for the duration of the measurement, it is likely that the movements of individuals will be masked to an extent. Finally, the filtering-out of *VV* values >50 mm s^{-1} needs to be justified. Given the composition of the mesozooplankton community (Table 4.5), it is likely that the fastest-moving particles contributing to the backscatter would have been *M. norvegica*, which is capable of swimming at speeds of up to ~100 mm s^{-1} (e.g. Tarling *et al.*, 1998). However, the fact that most of the *VV* values >50 mm s^{-1} were found in the surface layers during the day in the winter (Figure 4.13), where no krill were found (Figure 4.9), suggests that these higher speeds were not reflecting the movements of these animals. It therefore seems more likely that these high values were an artifact, caused by either (1) surface reflection, or (2) a lack of return signal due to low particle densities at the surface and/or high particle densities at depth causing strong sound absorption.

Mesozooplankton DVM behaviour and the potential for an active flux to occur

It is likely that *Calanus* and krill (*M. norvegica* and *T. raschii*) would each have been contributing to an active flux at one time or another in Inchmarnock Water as a

consequence of their DVM behaviour. Given that *Calanus* was primarily investigated during the present study, the following discussion relates to this species only.

The demographic strategy of Calanus

The net-catch data (Table 4.5) clearly showed that the *Calanus* population in Inchmarnock Water cycled through multiple generations over the course of the year. This multi-generational life-cycle has been observed in many of the past studies which have been carried out on *Calanus* throughout its main distributional range (see Marshall & Orr, 1955, p.64 for references), and may represent an adaptation to a vertically and temporally variable environment. Furthermore, the life-cycles of both *C. finmarchicus* and *C. helgolandicus*, when they co-exist in any one area, are usually found to be similar (Rees, 1949). Marshall & Orr (1955) described the life-cycle of *Calanus* in Loch Striven during 1933, and this has been taken to represent the typical sequence of events in the Clyde Sea Area. At this latitude, fertilised eggs mature inside the females for up to a month, while the timing of egg laying can be controlled (even delayed if necessary) to coincide with favourable environmental conditions. From egg to adult, the whole life-cycle (= 1 generation) takes about a month, after which the adults mate, spawn and then die. The annual life-cycle in Loch Striven begins in January/February, when deep-dwelling stage CV overwinterers moult and then copulate at depth (where the warmest water is often found). Females perform strong DVM during this time, while males remain at depth, and it is tempting to explain this dichotomy in terms of the different energy requirements of each sex (i.e. egg-bearing females needing to undertake feeding migrations in order to maintain their reproductive output). The first generation of the year is spawned in February/March, ahead of the spring diatom bloom, and grows up almost entirely in the surface layers until a second generation is spawned at the surface

in April/May. The apparent lack of DVM might suggest that visual predation pressure is not a factor at this time. The second generation develops throughout June, when DVM behaviour (albeit variable) is apparent, and a third generation is spawned in July. The bulk of the third generation arrests its development at stage CV in August, with only a small proportion moulting further into adults to spawning a fourth generation in September. The bulk of the population then remains at stage CV and overwinters at depth (>100 m), where individuals typically undergo physiological changes analogous to diapause in terrestrial insects (e.g. Leather *et al.*, 1993).

During the present study, it is therefore likely that *Calanus* samples taken in June/July would have consisted of second generation individuals, which might be expected to be variable in terms of their DVM behaviour, and that at least some of the females would have been gravid at this time. Samples collected in August probably consisted mostly of third generation individuals, some of which would have been preparing to overwinter at depth as stage CV, and some of which would have been preparing to moult and/or copulate within the next month. One might therefore have expected to see variable patterns of behaviour between individuals depending on their winter strategy. Samples from October are likely to have consisted of both third and now fourth generation individuals. One might not expect to see DVM behaviour in stage CV, while it is unclear what kind of behaviour to expect from stage CIV and adult females at this time of year. The overwintering third and fourth generations are also likely to have been sampled in December and January, when one might expect to see a general movement to depth and a reduction in DVM behaviour in view of the decreasing temperatures and (one assumes) availability of food. The increased numbers of adult males in mid-January, and the subsequent presence of stage CII in early March, suggests that arousal from diapause (assuming diapause had been initiated) and

subsequent moulting had taken place in January/February, followed by the spawning of the first, surface dwelling, generation of the year in late February. Evidence for another spawning at the beginning of May (indicated by the massive numbers of stage CII caught in May 2000) means that samples at this time were likely to have consisted of both first and now second generation individuals, at the generational changeover period between continuous residence at the surface and the onset of DVM.

The vertical migration strategy of Calanus

Both the net-catch (Figure 4.8) and acoustic data (Figures 4.12 and 4.13) indicated that the vertical distribution of *Calanus* in Inchmarnock Water varied over both diel and seasonal time scales, and both between and among developmental stages. This is not surprising given the reproductive strategy described above, and the variable nature of both the physical and biological environment. The net-catch data, in conjunction with the CTD data (Figure 4.5), indicated that there were times during the year when at least some of the population was likely to have been migrating through a pycnocline, thereby constituting interzonal DVM and the potential for an active flux to occur. These migrations, evidenced by noticeable differences in biomass in the surface layers between night and day, appeared to have been a feature in all sampling months except May. These day/night changes in biomass were most marked in August (stage CIV, CV and adult females) and October (stage CIV and adult females), indicating that, should it have been occurring, the active flux would have been most marked at these times. Given that the most pronounced stratification occurred in August ($\Delta\sigma_t = 2.5$ kg m^{-3}, see Figure 4.5), it is likely that the active flux had the potential to be the most significant at this time. While day/night changes in surface biomass were also apparent in December and January, the lack of a pycnocline at this time of year (December: $\Delta\sigma_t$ <0.1 kg m^{-3})

means that these movements were not strictly interzonal, and therefore would not have resulted in the sequestration of any surface-derived material at depth.

If these net tows were our only source of information, we might have concluded that the DVM behaviour of *Calanus* was relatively simple (and how much easier this discussion would have been!). However, additional net tows in August (Figure 4.11) revealed that the DVM of *Calanus* is likely to have involved a pre-dusk ascent. The acoustic data from June to December (Figures 4.14 and 4.16) confirmed this pattern and supported the idea that it was due to *Calanus*, given the *Sv* range during this period (–80 to –72 dB) and the interpretations given above (i.e. 'medium *Sv*'). This ascent, which may have been initiated as much as 8 h before sunset (Figure 4.14b), involved swimming speeds of up to 20 mm s^{-1}. Speeds of this magnitude, given that they are also likely to have been biased low (as discussed above), indicate that this pre-dusk ascent was a deliberate and synchronised event. One cannot discount the added possibility that some individuals were also undertaking a 'normal' dusk ascent within the SSL (Figure 4.12), at which time swimming speeds, at up to 10 mm s^{-1}, were slower than during the pre-dusk rise. The net-catch data were not able to resolve the timing of descent from the surface layers. However, the acoustic data for the period June to December detected what appeared to be a post-dusk downward dispersion (i.e. midnight sinking) of *Calanus* less than an hour after sunset (Figures 4.14 and 4.15), during which individuals may have descended to 60 m or more. The measured vertical velocities of up to 10 mm s^{-1} suggested that this midnight sinking involved a degree of active swimming (given passive sinking rates of <8 mm s^{-1}, see section 4.1.4). Furthermore, acoustic evidence for a pre-dawn rise during each of the sampling visits (Figures 4.14 and 4.16) hinted that, from June to December at least, interzonal DVM in *Calanus* involved the following pattern: (1) a pre-dusk ascent (up to 8 h before sunset) to feed in the surface

layers; (2) midnight sinking (within 1 h after sunset) to depths of 60 m or more; (3) a pre-dawn rise (1-2 h before sunrise) back to the surface layers; (4) a dawn descent. What this indicates, apart from the fact that the behaviour of this species is complex and therefore exceedingly hard to interpret with conventional sampling methods, is that individuals may have been crossing up and down through the pycnocline more than once during the diel cycle.

Similar observations have been made in previous investigations. For example, Gauld (1953) only detected clear evidence of migration in Loch Fyne on two occasions (July and November) during an 18 month study. Furthermore, having measured gut fullness in much the same way as during the present study, he found that animals at depth contained food. With no evidence for synchronised vertical movements by the whole population, and assuming (rightly or wrongly) that no food would have been available at depth, he concluded that the population "was in a continuous state of flux, some *Calanus* migrating upwards to feed and others downwards out of the rich water at all times of the day". Similarly, Simard *et al.* (1985), working in the St. Lawrence estuary, showed that *C. finmarchicus* reached the top 10 m just after sunset, began to perform midnight sinking within ~30 min, returned to the surface for a second period of feeding later on during the night and then descended at dawn. In support of both Gauld (1953) and the present study, they also agreed that "there was a dynamic interchange of individuals between the two strata, which masked the fact that all the copepods migrated to the upper stratum and that a dawn rise actually happened". However, contrary to the laboratory observations of Gauld (1953), they suggested that this vertical migration behaviour was linked to an *in situ* grazing rhythm.

In addition to these DVM patterns, two other patterns of behaviour were apparent. Firstly, during every sampling mission, the persistence in the net tows of at least some

individuals in the deep-water layer (>50 m) both day and night (Figure 4.8) was indicative that a variable proportion of the population was not undertaking DVM, remaining at depth even during the night. Furthermore, the fact that these deep-dwelling populations were not detected acoustically (Figure 4.12), except maybe as a continuous band of slightly increased backscatter (–70 dB) between 90 and 100 m in May, suggests that individuals may have been relatively diffuse and remained relatively still while at depth. Secondly, no DVM was evident from the net-catch data in May, given the fact that individuals were found to be almost equally distributed throughout the water column both night and day (Figure 4.18). If true, this observation would confirm the findings of Marshall & Orr (1955), namely that first-generation individuals of *Calanus* do not perform DVM. It is possible, however, that a continuous, asynchronous migration of individuals was being masked by the population-based information available from the net tows. Certainly a very close look at the acoustic backscatter data at this time (Figure 4.12f) might have shown a hint of a midnight sinking pattern. However, the more noticeable pattern of NDVM at this time is more likely to have been caused by the movements of krill, given that they were seen to contribute to a similar pattern in all other sampling months.

Causes of the vertical migration of Calanus

Are there any indications as to why *Calanus* behaved as it did? As Mangel & Clark (1986) discussed, there are a variety of theories to explain the behavioural ecology of animals, including those of optimal foraging, life-history strategy, territoriality, reproductive strategy and anti-predation and competition. In short, it is the need to reproduce, obtain energy for growth and avoid mortality while performing these tasks that are fundamental to the behaviour of any animal. Behavioural decisions may be

modified by factors such as the current state of the animal (e.g. lipid reserves, gut contents, body mass or body length), the current state of its environment, and maybe also on past and predicted future states (Cheverton *et al.*, 1985). Furthermore, according to Lima & Dill (1990) at least, it is the risk of predation which is the "determinant of [any animal's] behaviour from the outset". These factors are certainly reflected in the variety of theories that have been put forward to explain how and why zooplankton are stimulated to perform vertical migrations (see section 1.1.6). It might be suggested that the multi-generational reproductive strategy of *Calanus* has evolved as an adaptation to a vertically and temporally variable environment. Moreover, as an omnivore, one might expect *Calanus* to follow not only the distribution of its phytoplankton food source, but also that of its animal (metazoan) prey (which may be performing its own pattern of VM), all the while making the trade-off between feeding (and, ultimately, reproductive success) and the avoidance of predation (see e.g. Gerritsen, 1980).

Why, then, did *Calanus* need to ascend into the surface layers? The depth-profiles of chlorophyll *a* fluorescence from October 1999 to May 2000 (Figure 4.7b) indicated that the bulk of the phytoplankton food was to be found in the top 40 m of the water column, and in particular in the top 20 m. It is therefore likely that at least part of the reason why individuals ascended was to exploit the rich phytoplankton food-source at these depths. Note, however, that calanoid copepods typically prefer zones of high primary productivity to zones of high chlorophyll concentrations (e.g. Herman *et al.*, 1981), and that food quality may be more important than quantity (e.g. Kleppel, 1993). There is surprisingly little information in the literature on the metazoan food of *Calanus*, making it hard to know if any of the mesozooplankton species sampled here (>300 μm) represented a potential food source. Lebour (1922) identified parts of *Paracalanus parvus* in the stomach of *C. helgolandicus*, making it possible that some of the other

small mesozooplankton species that were caught, for example *Oithona* spp., *Evadne* spp., *Podon* spp., fish eggs and various larvae, might also have been utilised as food. Also, Petipa (1965) spoke of *C. helgolandicus* biting the heads and tails off slow-moving rockling larvae in the Black Sea, making it feasible than fish larvae were a potential food source for *Calanus* in the Clyde Sea. For the most part, these potential mesozooplankton prey were to be found in the surface layers (<50 m), although some species certainly appeared to descend to depths of up to 130 m at times (Figure 4.10). Their predominance towards the surface, however, provides another reason as to why *Calanus* might need to spend time in the top 50 m of the water column. Recalling the gut fullness observations in March and May 2000 (Figure 4.25), it was shown that individuals at depth during the day had food material in their stomachs, but that it was unclear as to whether this food had been ingested at the surface or at depth. Either scenario is possible, given that metazoan prey and/or detritus may have been available at depth. However, the fact that at least a proportion of the *Calanus* population was observed to invest time and energy in swimming toward the surface might suggest that, while food may well have been available at depth, it was not sufficiently nutritious to maintain a healthy state for any length of time.

Having established that the upward migrations of *Calanus* enabled feeding in the food-rich surface layer, we can ask why downward migration (both midnight sinking and dawn descent) out of the surface waters was necessary. The most widely accepted reason is that the risk of predation for such an animal is higher near the surface (see section 1.1.6). Certainly, one might expect that dawn descent occurred to enable the avoidance of visually orienting fish, which are known to inhabit these waters and predate on *Calanus*. However, a variety of other, proximate, causes have been put forward to explain midnight sinking. Cushing (1951), for example, suggested it to be

the result of a general slowing of swimming speeds as light levels fall, while Gauld (1953) and Pearre (1973, 1979b) suggested that satiated individuals might either swim slower and therefore sink (e.g. Mackas & Bohrer, 1976), or simply be denser following feeding (e.g. Krause & Radach, 1989). One way to establish that predation pressure was a factor would be if we could see some sort of correlation between the behaviour of predators and prey. Strikingly, this was seen to be the case with the krill species *M. norvegica* and *T. raschii*, both of which are potential predators on *Calanus* (e.g. Mauchline, 1959; Bamstedt & Karlson, 1988; Lass *et al.*, 2001). In August 1999, there was a particularly noticeable temporal correlation between the arrival of predatory krill at the surface just after dusk, and the midnight sinking of *Calanus* (see Figures 4.8, 4.9 and 4.12b). It is therefore feasible that *Calanus* was performing a pre-dusk ascent followed by midnight sinking in order to maximise the time spent feeding on what was likely to have been more nutritious food in the surface layer (see discussion above), while minimising the time spent in contact with predatory krill (see Tarling *et al.*, 2002, for further discussion of these data). While one might have expected the cover of darkness to afford *Calanus* some protection from visually orienting krill, a number of studies have shown that krill may also act as tactile predators (e.g. Wright & O'Brien, 1984; Torgersen, 2001). A similar pattern of avoidance was suggested in the northeast Atlantic by Heywood (1996), who found that one SSL ascribed to copepods left the surface layer upon the arrival of another SSL ascribed to krill.

This feeding/predator-avoidance trade-off might also mean that individuals would have been moving into and out of the surface layer more than once during the course of the night. This idea is supported by the observations of a bimodal gut fullness distribution in the surface layer at night (Figure 4.25), which might have suggested that the guts were being filled and emptied more than once during this period. Given that an

individual with full guts may be more visible, and hence more susceptible to predation (e.g. Tsuda *at al.*, 1998), than one with empty guts, one might expect a retreat to depth between feeding bouts in order to digest and assimilate. Assuming a gut-filling time (GFT) of 30-60 min (e.g. Gauld, 1953; Mauchline, 1998), and a gut-passage time (GPT) of 30-100 min (from Figure 4.24: this estimate also agrees with that of Gauld, 1953), an individual will be able to fill its stomach relatively rapidly at the surface, digest this food over the course of an hour or so in the relative safety of the depths, and then return for a second meal. The number of times an individual could perform this would depend on the length of the night and the individual's rates of digestion and assimilation. Interestingly, this behaviour might be said to provide evidence for both the predator-avoidance hypothesis (e.g. Zaret & Suffern, 1976) and the hunger/satiation hypothesis (see Pearre, 2003) at the same time. While *Calanus* individuals are highly likely to be performing midnight sinking to avoid predation, the amount of exposure an individual will tolerate may well be dictated, at least in part, by hunger and satiation. In order to decide the hierarchy of this decision-making process, one would need to find out whether individuals were truly satiated upon descent, or whether the risk of predation had initiated descent before satiation was reached.

Krill are not the only predators on *Calanus* in the Clyde Sea. Other potential predators include ctenophores, medusae, chaetognaths, carnivorous copepods (e.g. *Euchaeta norvegica*) and fish (e.g. herring). The behaviour of *Calanus* may therefore be influenced by "complex antagonistic relationships with multiple types of enemies" (Decaestecker *et al.*, 2002). The likely presence of different predators such as fish in the surface layer during the day might explain why *Calanus* does not simply perform RDVM (e.g. Ohman *et al.*, 1983) and remain feeding at the surface at this time. If one assumes that larger individuals are more susceptible to visual predators (e.g. Hays *et al.*,

1994; Thetmeyer & Kils, 1995; de Robertis, 2002), this would also explain why the larger stages (stage CIV and larger) needed to take refuge in the darker depths, while the smaller stages (stage CIII and smaller) could remain at shallower depths (Figure 4.8) where the feeding was assumed to be better. Evidence as to whether or not *Calanus* might also need to avoid extended exposure to the sunlit surface layer due to the harmful effects of UV is ambiguous: while sunlight has been found to be lethal to *Calanus* (e.g. Marshall *et al.*, 1935), individuals are known to frequent the surface layer during the day, even during bright sunshine (e.g. Bainbridge, 1952). The likelihood that individuals had been feeding during the day (Figure 4.25) suggests that the nighttime trade-off with krill did not allow sufficient feeding to occur during the hours of darkness. The distance to the surface layer was not so great as to preclude the possibility of daytime feeding sorties from depth, followed by satiation- and/or predation-mediated descent. The fact that individuals were distributed throughout the whole water column (Figure 4.8), and the fact that the fullest individuals were found closer to the surface, would certainly support this theory. However, this pattern may also have been a function of a depth-related gradient in food availability, and the possibility that detritivorous and/or carnivorous feeding had been occurring at depth cannot be ruled out (recall, however, the suggestion above that food quality at depth may well have been lower than that at the surface).

Regarding continuous residence at depth, Hays *et al.* (2001a) found that a proportion of the *Metridia pacifica* population in Dabob Bay, Washington State, remained at depth during the night, and showed that deep-dwelling individuals had larger oil-sacs than migrants found in the surface layers. They proposed that individuals with larger oil-sacs (hence, better body condition) did not need to feed as often as those with smaller oil-sacs, and therefore did not need to migrate to the surface layer every night where the

risk of predation was assumed to be higher. Their "rough and ready" calculation showed that deep-dwelling individuals could avoid the surface layers for up to 9 d before energy reserves fell to a threshold level and DVM needed to be resumed. Conversely, in the most suitable instance during the present study where stratified nighttime samples were available (December 1999), the carbon weight of size-normalised (2.18 mm-long) surface-caught (0-15 m) individuals was actually higher than that of deep-caught (100-130 m) individuals (mean±1SD: 141±16 versus 113±41 μg C ind^{-1}), as was C:N (mean±1SD: 9.8±0.2 versus 7.1±1.6) and C:length (mean±1SD: 63±9 versus 51±20 μg C mm^{-1}). The fact that 'body condition' was therefore actually better (in terms of carbon per unit body length) in individuals that were caught closer to the surface indicates that the reasons for deep-residence in this case were not the same as those in Dabob Bay. However, it is hard to explain this observation in terms of the known overwintering strategy of *Calanus*, given that one might have expected individuals at depth in December to have greater lipid reserves than those still performing migrations (see e.g. Ingvarsdottir *et al.*, 1999). In fact, it appears as though the opposite was true. One explanation is that two different overwintering strategies were employed. Those individuals still migrating into the surface layer might have been able to feed more successfully than those that remained at depth, thereby increasing both their body condition and their chances of surviving the winter. However, one can only speculate on their potential food source at this time. Other studies in which continuous deep residence has been observed include those of Tarling *et al.* (1999b) for moulting *M. norvegica*, and Bollens & Frost (1991) for egg-bearing *Euchaeta elongata*, demonstrating that there are a variety of different reasons as to why individuals might temporarily cease migrating and remain at depth. As Hays *et al.* (2001a) discussed, predictions of migration behaviour influenced by body condition (e.g. Sekino &

Yamamura, 1999) have "been tested with little empirical evidence", and this would certainly be an interesting area for future study.

4.4.3 Was an active flux occurring, and could it be measured with the current dataset?

The discussion above has described what we have learned about the physical environment of Inchmarnock Water, and the composition and behaviour of the mesozooplankton community. Based on this information, the main focus of this study can now be addressed: would an active flux have been occurring in Inchmarnock Water, and could this potential flux have been successfully measured with the present dataset?

Evidence for flux-conducive behaviour

Given that krill (*M. norvegica* and *T. raschii*) were seen to perform strong NDVM during this study (Figure 4.9), one might expect that they would have been major contributors to an active flux during the stratified summer months. However, as discussed above, the possibility that daytime benthic feeding could have been taking place means that this contribution may well have been diminished. It is most unfortunate that the body-weight data for these species were lost, so that the dawn-dusk difference in carbon and nitrogen weight could not be assessed. Indeed, this is the reason why these species have not received the same amount of attention here as *Calanus*. The general uniformity of their DVM behaviour means that there was a good chance that the samples would have been collected at the critical times of movement, such that the data may well have provided a good representation of the true daily losses of carbon and nitrogen at depth. Unfortunately, one can only speculate at present as to the importance of krill to the active flux in Inchmarnock Water.

When considering *Calanus*, it has been shown above that the DVM behaviour of at

least parts of the population at various times was capable, in theory, of exporting material from the surface layers to the depths, where it may have been sequestered for a time. Most importantly, the possibility that individuals were crossing the pycnocline more than once during the diel cycle implies that the active flux may well have been greater than if a classic pattern of NDVM was being performed. This idea has also been suggested by Pearre (2003), who, in discussing *Calanus finmarchicus* in the North Atlantic, wrote that, "if it is in fact migrating asynchronously, but undetectably, driven by hunger and satiation...its contribution to both carbon and nitrogen fluxes could be very large". Moreover, the indication that a single gut-full of material may have contained as much as 15 % of the carbon, and 24 % of the nitrogen weight of an empty individual (see Figure 4.19), suggests that defaecation at depth could have been an important contributor to the active flux, especially if, after every feeding bout at the surface, individuals descended immediately below the pycnocline. Pearre (2003) discussed the consequences of less time for defaecation in the surface waters (after the field observations of Gibbons, 1993): "this may relax some of the constraints on migrator size required for meaningful contribution to the flux (see Tseytlin, 1982, 1999; Longhurst *et al.*, 1989; Lampitt *et al.*, 1993), as well as meaning that large migrators would also transport a greater proportion of their near-surface ingestion than previously supposed".

Quantifying the active flux according to ZOOFLUX

Although the DVM of zooplankton in Inchmarnock Water probably resulted in an export of carbon and nitrogen to depth, the current dataset was unable to quantify this flux directly with the ZOOFLUX technique (see section 4.3.4). There are a variety of methodological and biological reasons for this. Firstly, in terms of methodology, the

sample sizes were relatively low, for reasons already given, and this impacted on the statistical power, or success, of the ZOOFLUX technique (Table 4.12). In an attempt to reinforce the sample numbers, carbon and nitrogen weights were derived from dry weight measurements in many cases (represented by open symbols in Figure 4.27). However, despite the apparently strong linear relationships between dry weight and both carbon and nitrogen weight (Figure 4.20), it is evident that these may not be a reflection of true biological relationships, and one might therefore express misgivings about using dry weight as a proxy (especially for nitrogen, which is less representative of body bulk than carbon). Secondly, in terms of biology, the complex and highly variable behaviour of *Calanus* at this site as revealed by acoustic and net sampling methods, while fascinating from a behavioural ecologist's standpoint, generated uncertainties as to the nature of the information available from the biometric data. Specifically, the multi-generational demographic strategy, and a vertical migration strategy influenced by a multitude of environmental factors, means that individuals possibly exhibiting radically different behavioural strategies, and maybe even representing different generations, may have been present at the same depth in the water column at the same time. This made it particularly difficult, if not impossible, to obtain the correct type of samples for the successful application of ZOOFLUX. In short, low sample numbers and high ecological variability impacted on the ability to quantify any active fluxes potentially caused by the DVM behaviour of *Calanus* in Inchmarnock Water.

Linear regression analysis of the biometric parameters provided interesting insights into the ecology of individuals. The observation that the length/weight body condition of individuals from different depths, times of day, seasons and developmental stages was fundamentally similar (Figure 4.18) is interesting, and hard to explain, given the highly variable behaviour of *Calanus* inferred from the net tows and acoustics. Indeed,

the only deviation from this pattern was exhibited by stage CV individuals preparing to overwinter (Figure 4.18b). One indication from this is that, with the exception of these carbon-rich overwinterers, length/weight body condition would not have been a causal factor in the VM behaviour of individuals. On the other hand, the large amount of variability in the nitrogen/carbon body condition of individuals (Figure 4.21) might suggest that differences in the relative proportion of lipids to proteins may have had a causal effect on VM. While the data do not lend themselves to a comprehensive test of this hypothesis, given the general lack of concurrent samples from different depth strata, where such samples were taken in December 1999 it was found that it was the more lipid-rich individuals which were undertaking DVM. Of course, this may not have been the case at other times of the year.

It therefore seems likely that the consistent lack of a dawn-dusk decrease in the carbon and nitrogen content of *Calanus* collected in the field at different times of the year (Figure 4.27) was due to its variable behaviour. The inability to detect noticeable changes even in experimentally starved individuals (Figure 4.26) might suggest that individual variability is high even in individuals behaving in the same way. However, given that a positive relationship was found between gut fullness and body weight (Figure 4.19), and that significant decreases were recorded in the gut fullness of starved individuals (Kruskal-Wallis: $P = 0.006$, Figure 4.24), it is difficult to explain why such reductions were not also detected in the carbon and nitrogen weight of these individuals. One might argue that, given the inability to detect body weight changes in experimental animals, there was little point in attempting to detect the more subtle and complex changes likely to be occurring in the field. This is certainly a lesson to bear in mind for any future such studies. Furthermore, given the way in which the *Calanus* population was behaving (e.g. pre-dusk ascent, midnight sinking, pre-dawn rise, continuous

residence at depth etc.), it is hardly surprising that consistent changes were not detected in the field-sampled individuals.

In conclusion, while the incredible behavioural plasticity of *Calanus* suggests that it is supremely adapted to survival in the pelagic environment (hence its great abundance throughout the North Atlantic), and a fascinating animal to study from a behavioural ecology perspective, this trait does not render it an appropriate organism for the execution of ZOOFLUX.

4.4.4 Summary: the active flux in Inchmarnock Water

1. The physical environment exhibited seasonal changes (stratification in June and August 1999, mixing in October and December 1999, and restratification in March and May 2000).
2. The greatest rates of relative change in light intensity occurred around first and last light during each sampling mission.
3. Levels of chlorophyll *a* exhibited seasonal changes (summer peaks in June and August 1999, autumn pulse in October 1999, the end of the spring bloom in March 2000, and summer peaks in May 2000).
4. The *Calanus* population may have cycled through as many as four generations over the course of the year. Krill appeared to recruit twice during the year, in spring and late summer.
5. *Calanus* exhibited a complex variety of vertical migration patterns. Krill (*Meganyctiphanes norvegica* and *Thysanoessa raschii*) underwent a more synchronous pattern of NDVM.
6. Third and fourth generation *Calanus* may have undertaken one of two overwintering strategies: (1) continuous residence at depth in a state of diapause, or (2) a continuing

pattern of DVM and feeding. The krill population was subject to severe mortality during the winter months.

7. The body condition (length:weight) of *Calanus*, which was relatively uniform for most of the year, improved during the winter months.
8. Defaecation may have represented a potentially significant avenue for carbon and nitrogen loss in *Calanus* at certain times of the year.
9. For *Calanus*, there was no evidence that smaller individuals within any given stage were to be found closer to the surface, or *vice versa*.
10. *Calanus* collected in the field at different times during the diel cycle showed evidence for a diel feeding rhythm, feeding at higher levels during the hours of darkness, and lower levels during the day.
11. *Calanus* starved in laboratory incubations were found to void their guts within 100 min. However, no systematic decreases in body carbon or nitrogen weight were detected in these individuals.
12. For *Calanus* collected in the field at different times during the diel cycle, δ was found to be variable and V relatively high, while n was relatively low. The behavioural plasticity of *Calanus* at this site does not render it an appropriate organism for the successful application of ZOOFLUX.

5

LONG-DISTANCE VERTICAL MIGRATIONS IN THE OPEN-OCEAN: *PLEUROMAMMA XIPHIAS* (CRUSTACEA: COPEPODA) AND KRILL (CRUSTACEA: EUPHAUSIACEA) IN THE SARGASSO SEA

5.1 Introduction

5.1.1 A history of research in the Sargasso Sea

"The blue which filled all space admitted no thought of other colours"

William Beebe, "Half Mile Down" (1934)

The Sargasso Sea near Bermuda (Figure 5.1) is "one of the most heavily documented oceanic environments in the world" (Michaels & Knap, 1996). This is no doubt due, at least in part, to the availability since 1903 of laboratory resources at the Bermuda Biological Station for Research (BBSR), and the relative ease of access to the open-ocean environment. In 1954, the Hydrostation S time-series programme was initiated at a site (also known as the Panulirus Station) 26 km southeast of Bermuda (Schroeder & Stommel, 1969). The focus of this ongoing programme has been the fortnightly measurement of temperature, salinity and dissolved oxygen from the whole water column (0-3000 m), and this is now the world's longest continuous open-ocean time-series. Measurements from this site have proved invaluable in helping to explain changes in the physical structure of the ocean on timescales from months to decades (e.g. Schroeder *et al.*, 1959; Schroeder & Stommel, 1969; Pocklington, 1972; Wunsch, 1972).

From 1957 to 1963, there was a concerted research effort in the region. Measurements of nutrients, chlorophyll *a* and primary productivity were temporarily added to the Hydrostation S programme (Menzel & Ryther, 1960a, 1961a), while a variety of other studies were also carried out. These included studies of phyto- and zooplankton seasonality (Be, 1960; Hulbert *et al.*, 1960; Chen & Be, 1964; Deevey, 1968), zooplankton biology (Sutcliffe, 1960; Menzel & Ryther, 1961b; Beers, 1964, 1966; Deevey, 1964), nutrient distributions, interactions between nutrients, plankton

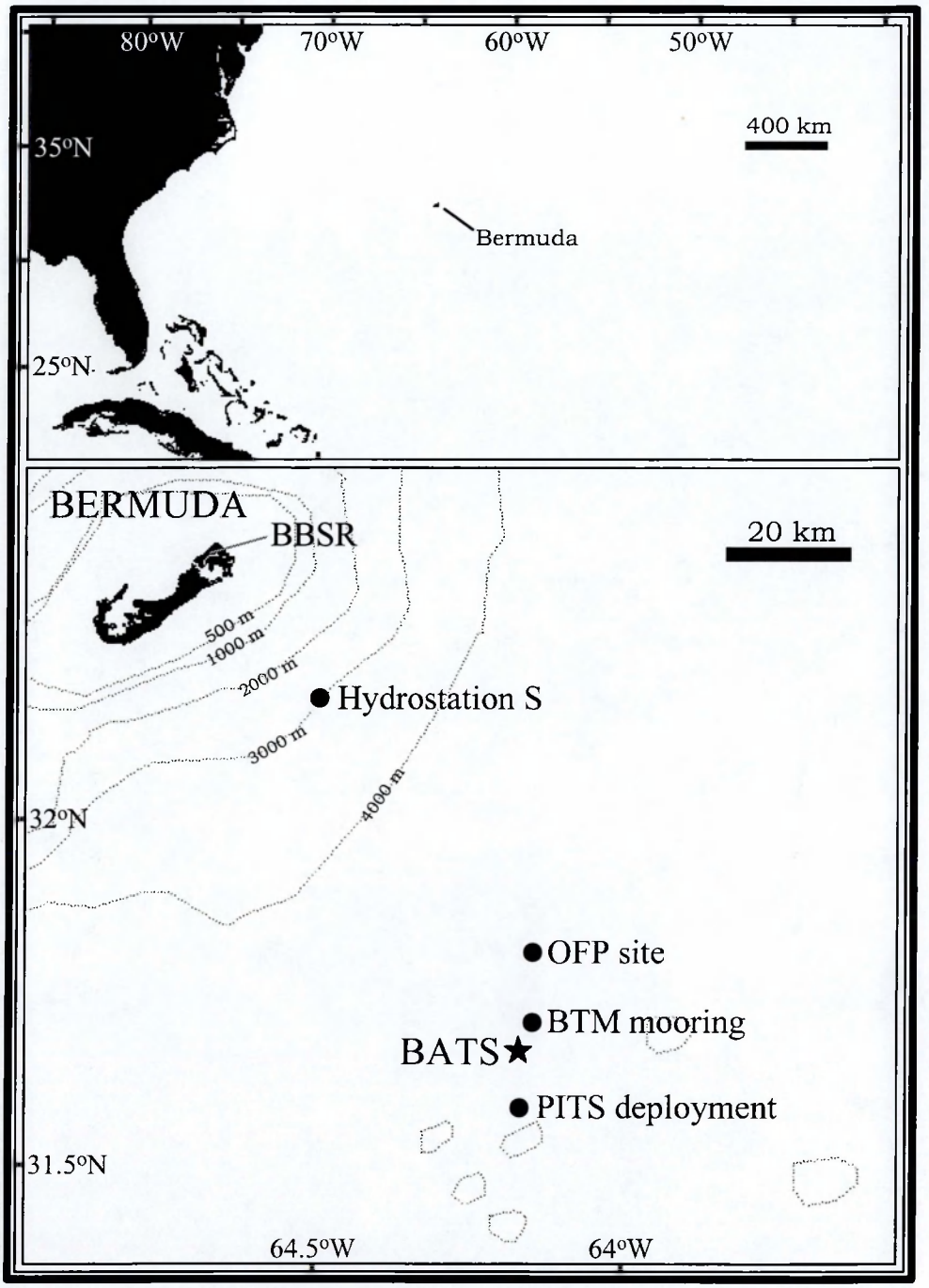

Figure 5.1 The position of Bermuda and a number of oceanographic sampling sites in the Sargasso Sea. BATS = Bermuda Atlantic Time-series Study site. BTM = Bermuda Testbed Mooring. PITS = Particle Interceptor Traps (sediment traps). OFP = Ocean Flux Program. BBSR = Bermuda Biological Station for Research.

and productivity, iron limitation, light in relation to production, and marine aggregates (Riley *et al.*, 1965; Wiebe & Pomeroy, 1972). Further studies on zooplankton dynamics were also made in the late 1960s (Deevey, 1971; Deevey & Brooks, 1971).

1976 saw the inception of what is now the world's longest ongoing deep-ocean particle-flux programme. A sediment trap was deployed and recovered every two months, initially at 3200 m depth at a site just to the south of Hydrostation S (Deuser & Ross, 1980; Deuser, 1986). Later, this site was moved further offshore to what is now known as the Ocean Flux Program (OFP) site (Conte *et al.*, 2001). At various times, these samples have also been used to study trace element fluxes, fly ash, foraminifera, pteropods and radionuclides. Following the recognition that air-sea exchanges may prove to be important in upper-ocean processes, daily measurements of atmospheric pollutant transport were begun in 1980. A BBSR-managed site and a measuring tower run as part of the Aerosol Oceanic Chemistry Experiment (AEROCE) are still active on Bermuda today.

Since 1982, fortnightly measurements have been made of dissolved inorganic carbon (DIC) in surface seawater samples from Hydrostation S (Keeling, 1993). From 1984 to 1989, a time-series study of the upper ocean nitrogen cycle was carried out every two months near to the OFP site (Altabet, 1988, 1989a, b). From 1986 to 1994, studies of benthic boundary layer fluxes were made using an autonomous lander known as ROLAID (Sayles & Dickinson, 1991).

Following particularly extreme weather in North America in 1982-1983, public pressure prompted more research into the El Niño phenomenon. This cast the spotlight on the important role of the world's oceans in climate change. A major response by the scientific community was the inception of the International Geosphere-Biosphere Program (IGBP) in 1986. A core component of this programme was the Joint Global Ocean Flux Study (JGOFS), which was established in 1987 with the aim "...to determine and understand...the processes controlling the time-varying flux of carbon and associated biogenic elements in the ocean" (SCOR, 1987). Also in 1987, the World

Ocean Circulation Experiment (WOCE) was established, as part of the World Climate Research Program (WCRP), to address the role of the ocean circulation in the world climate system.

In 1988, under the research umbrellas of JGOFS and WOCE, the US National Science Foundation (NSF) funded two oceanographic biogeochemistry time-series studies: the Bermuda Atlantic Time-series Study (BATS) in the North Atlantic Ocean near Bermuda, and the Hawaii Ocean Time-series (HOT) in the North Pacific Ocean near Hawaii, both of which are ongoing today. The BATS study site is situated 82 km south-east of Bermuda, about 8 km south of the OFP site, where the water depth is ~4680 m. The core oceanographic measurements at BATS, which are made fortnightly to monthly by technicians at BBSR, are discussed further in section 5.2.2.

A wealth of ancillary projects has been carried out as part of, or in association with, the BATS programme (see Table 1 in Michaels & Knap, 1996). Other time-series studies that have been established following the inception of the BATS programme include the Bermuda Bio-Optics Project (BBOP) (1992-present) and the Bermuda Testbed Mooring (BTM) (1994-present). BBOP uses remote sensing techniques and *in situ* light measurements to monitor changes in biogeochemical cycles and to characterise and predict primary production rates (Siegel *et al.*, 2001), while the BTM is a deep-sea mooring that allows the long-term testing of oceanographic equipment (Dickey *et al.*, 2001).

5.1.2 Sargasso Sea hydrography

Bermuda is situated in the subtropical gyre of the western North Atlantic Ocean. This region is also known as the Sargasso Sea, after the pelagic macro-alga *Sargassum*

muticum ("Japanese Weed") that is frequently encountered here*. With the NE-flowing Gulf Stream to the north and west, and the W-flowing North Atlantic equatorial current to the south, the Sargasso Sea is a region of weak geostrophic recirculation (<5 cm s^{-1}) with a net SW flow (Siegel & Deuser, 1997). Mesoscale features such as cold core rings (horizontal scale 100-200 km) (e.g. The Ring Group, 1981) and other smaller cyclonic and anti-cyclonic eddies (horizontal scale 10-100 km) (e.g. McGillicuddy *et al.*, 1998; Siegel *et al.*, 1999) are common. The centre of the gyre is also characterised by net Ekman downwelling, with flow rates near BATS of ~4 cm d^{-1} (McClain & Firestone, 1993).

At 31.7 °N, BATS lies at the northern edge of a transition zone between relatively eutrophic waters to the north and relatively oligotrophic subtropical waters to the south. In the summer, most of the Sargasso Sea is influenced by the Bermuda-Azores high pressure system, and there is strong thermal stratification of the water column from April to October. To the north in autumn and winter, the regular passage of low pressure systems from North America breaks down the seasonal thermocline, allowing deep convective mixing and subsequent nutrient enrichment of the surface layer to occur (e.g. Worthington, 1976; Woods & Barkman, 1986). The depth of the mixed surface layer at this time may extend to as much as 400 m, forming what is known as the subtropical mode water (STMW) (Talley & Raymer, 1982). Recent research has shown that the formation of the STMW to the north of BATS may be producing a significant transport

* The name *Sargassum* derives from the Spanish word *sargazzo*, which signifies kelp. Old legends about the Sargasso Sea talk of vast mats of weed which entangled boats. As Jules Verne wrote in *Twenty Thousand Leagues Under the Sea*, the Sargasso Sea was "a perfect meadow, a close carpet of seaweed, fucus and tropical berries, so thick and so compact that the stem of a vessel could hardly tear its way through it". Such dense aggregations of *Sargassum* do not appear to be found these days – certainly fieldwork was not hampered during this study...

of atmospheric CO_2 to depth, although episodic features, such as mesoscale eddies and hurricanes, may penetrate this layer and return this sequestered carbon relatively rapidly (N.R. Bates & C. Pequignet, pers. comm.). To the south in winter, where there is generally less atmospheric forcing, the mixed layer rarely extends below 100-150 m (e.g. Malone *et al.*, 1993).

Following its winter formation, the ~18 °C STMW sinks beneath the seasonal thermocline, forming a relatively homogeneous zone (in terms of temperature) at BATS between 250 and 400 m depth (e.g. Halliwell *et al.*, 1994). It is this zone that has the greatest effect on the development of the mixed layer. Depending on the amount of winter cooling, the mixed layer may only extend to 100-200 m, hardly penetrating the nutrient-rich thermocline, or it may extend as deep as 300-400 m, resulting in significant nutrient input to the euphotic zone. These processes are, therefore, particularly important within the context of vertical export fluxes at BATS.

The levels of atmospheric forcing and cooling in the winter are responsible for much of the interannual variability at BATS. In years of low storm activity and warmer winters, the water column is similar to the permanently stratified oligotrophic waters to the south. The winter mixing depth is reduced, the surface waters are nutrient (nitrate) depleted, and the ecosystem is dominated by picoplankton and the microbial food web. In years of greater atmospheric forcing and winter cooling, the water column is more similar to the eutrophic waters to the north. The mixed layer extends deeper during the winter and becomes enriched with nutrients. Larger phytoplankton such as diatoms and coccolithophores are able to bloom in late winter/early spring (January-March), followed by a return to oligotrophic conditions in the summer.

5.1.3 Primary production and phytoplankton at BATS

Phytoplankton community structure

The phytoplankton community structure in the top 250 m at BATS has been described by Steinberg *et al.* (2001). As well as providing a review of past studies in the region, they determined the "signature" pigments within depth-discrete samples using high-performance liquid chromatography (HPLC), and used algorithms (after Letelier *et al.*, 1993) to infer the contributions of specific taxa to the deep chlorophyll maximum layer (DCML). Chlorophyll *a* concentrations were shown to peak typically between 60 and 120 m, thereby defining the extent of the DCML, although short-term surface peaks were also often observed in the winter following the convective mixing of deep (>100 m) mixed layers. Chlorophyll *b* concentrations were usually found to correspond with those of chlorophyll *a*. These methods revealed the presence of both prokaryotic (mainly picoplankton) and eukaryotic phytoplankton at BATS, with prokaryotes often dominating the community.

Of the prokaryotes, prochlorophytes (e.g. *Prochlorococcus* sp., *Synechococcus* sp.) are often abundant from late spring to early winter, and have been found to thrive to greater depths than the other prokaryotic picoplankton. Cyanobacteria (e.g. *Trichodesmium* spp.) are significant year-round, especially in late spring and during the summer. Their signature pigment, zeaxanthin, is often found in high concentrations at the surface during spring blooms, although, year-round, concentrations are generally highest between 40 and 120 m. Of the eukaryotes at BATS, prymnesiophytes are the most abundant (mostly larger coccolithophores). *Emiliana huxleyi* often dominates the spring bloom in May, while *Umbellosphaera* spp. and *Florisphaera profunda* predominate during the smaller bloom in early autumn. Their signature pigment, 19-hexanoyloxyfucoxanthin, is found predominantly between 30 and 120 m, where it

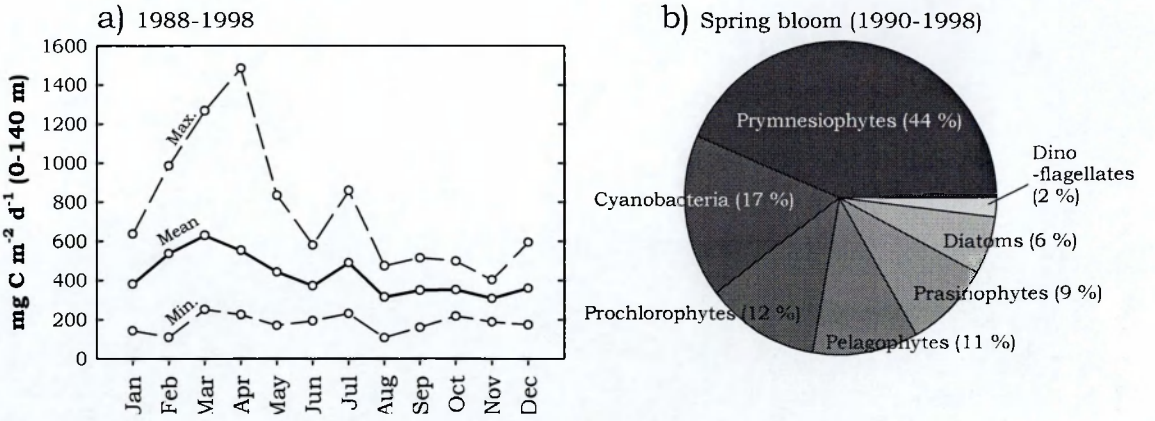

Figure 5.2 The annual cycle of primary production and the composition of the spring bloom at BATS. a) 0-140 m integrated daily ^{14}C uptake for each month (mean±range) during the period December 1988 to April 1998. Data courtesy of the BATS data repository at URL: http://www.bbsr.edu. b) The major phytoplankton taxa composing the spring bloom. Percentage values are means for the period 1990-1998. Data from Steinberg *et al.* (2001).

provides a significant contribution to the DCML. Pelagophytes (e.g. silicoflagellates) are also common, with their signature pigment, 19-butanoyloxyfucoxanthin, exhibiting a similar vertical distribution. Levels of diatoms (e.g. pennate species such as *Nitzschia* spp., and centric species such as *Thalassiosira* spp., *Coscinodiscus* spp., *Chaetoceros* spp. and *Rhizosolenia* spp.) are generally low. Blooms are rare and short-lived, with occasional peaks occurring during late or non-spring bloom periods. Their signature pigment, fucoxanthin, is present at all depths down to ~200 m at these times. Dinoflagellates (e.g. *Ornithoceros* spp., *Dinophysis* spp. and *Prorocentrum* spp.) are minor contributors to the phytoplankton at BATS, with low abundance at all times. When present, their signature pigment, peridinin, never reaches below ~150 m.

Primary production

Figure 5.2a shows the typical annual cycle of primary production at BATS, which exhibits peaks in spring (February to April) and late summer/early autumn (June to

August). Figure 5.2b shows the typical composition of the spring phytoplankton bloom. The bloom usually begins with an increase in the numbers of diatoms, after which a more diverse community of prymnesiophytes, cyanobacteria, prochlorophytes, dinoflagellates and diatoms develops. Prymnesiophytes tend to dominate during spring/early summer, being succeeded by cyanobacteria and prochlorophytes from late spring through to winter. Prasinophytes, dinoflagellates and pelagophytes (the dominant of these three groups) are present year-round. The relative composition of the phytoplankton community, especially the eukaryotes, does not seem to be affected strongly by winter mixing and spring/summer stratification, and growth rates do not appear to change with the seasons (Goericke, 1998; Goericke & Welschmeyer, 1998). According to Steinberg *et al.* (2001), this suggests a strong resilience to physical forcing by the phytoplankton at BATS.

5.1.4 Sinking particle fluxes at BATS

Steinberg *et al.* (2001) have shown that sinking particle fluxes at BATS are generally highest in the spring, but that peaks may also be observed throughout the year (Figure 5.3). However, they also showed that, for the period 1988-1998, there was a surprisingly weak correlation between sinking flux and primary production ($r^2 = 0.16$), even when a 1 week time-lag was incorporated to take into account mean particle sinking rates (50-100 m d^{-1}). A stronger correlation was found between particle flux and the depth of mixing. Deep mixing events, either seasonal due to wintertime atmospheric forcing, or episodic due to mesoscale features, were seen to coincide with peaks in measured particle flux. These findings were attributed to bias from sampling artifacts caused by the physical dynamics during these mixing events. This bias is being addressed through the continued development of new sediment trap designs (e.g. Buesseler *et al.*, 2000;

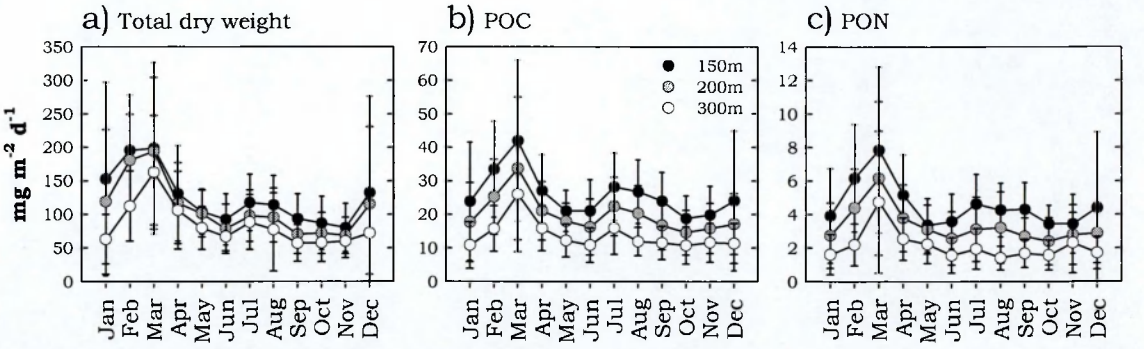

Figure 5.3 The annual cycle of sinking particle flux at BATS as measured from surface-tethered sediment traps at depths of 150, 200 and 300 m. Each point represents a mean (±1SD) monthly value for the period 1989-1998 inclusive (1-3 replicates per depth per month per year). a) Total dry mass, b) Particulate organic carbon (POC), c) Particulate organic nitrogen (PON). Data courtesy of the BATS data repository at URL: http://www.bbsr.edu.

Valdez & Price, 2000).

Lohrenz *et al.* (1992) and Steinberg *et al.* (2001) have shown that particle fluxes at BATS typically represent only a fraction of the geochemical estimates of total organic matter export in the region (made by Jenkins & Goldman, 1985). Furthermore, Steinberg *et al.* (2001) have shown that POC fluxes at 150 m are generally only a small fraction of the integrated primary production (0-140 m), with a low overall mean export ratio (POC flux/production) of 0.07 (range 0.02-0.22). These observations suggest that passive sinking fluxes, while still causing a vertical export of material from the surface layers, may not be as important at BATS as traditionally expected for an open-ocean ecosystem (see section 1.2.4). Furthermore, if one assumes that material fixed from primary production is not accumulating *ad infinitum* in the euphotic zone, it stands to reason that there must be other vertical and/or horizontal export fluxes at work.

5.1.5 Sargasso Sea zooplankton

Earlier studies in the Bermuda region

Records of zooplankton sampling in the Sargasso Sea near Bermuda date back at least 70 years (Leavitt, 1935, 1938; Clarke, 1940; Riley & Gorgy, 1948), but these early studies, made from oceanographic cruises that happened to be crossing the area, were somewhat opportunistic and therefore restricted in both space and time. More detailed studies that have been undertaken in the region include those of Moore (1949) from the "Bermuda area", Fish (1954) from oceanic station E (located some distance ENE of Bermuda), Grice & Hart (1962) from a transect station to the NW of Bermuda, and von Bodungen *et al.* (1982) from station R (located 7-11 km SE of Bermuda). Studies at Hydrostation S have primarily involved daytime net tows from the surface to 500 m (Sutcliffe, 1960; Menzel & Ryther, 1961b; Deevey, 1964, 1968, 1971; Beers, 1966; Deevey & Brooks, 1977), while only a few investigators have deployed nets to below 500 m (Menzel & Ryther, 1961b; Deevey, 1964; Deevey & Brooks, 1971).

The mesozooplankton community

Reviews of zooplankton at BATS have been published by Madin *et al.* (2001) and Steinberg *et al.* (2001). Studies have shown that the bulk of the heterotrophic carbon-biomass in the euphotic zone at BATS (~70 %) is made up of bacterioplankton and nanozooplankton (= protozoa 2-5 μm) (Roman *et al.*, 1995; Caron *et al.*, 1995), and that relatively little is passed on to the metazoa (Caron *et al.*, 1999). Nutrients therefore predominantly pass through what is known as the "microbial loop" (Azam *et al.*, 1983; Hobbie & Williams, 1984). Despite this, it is the larger organisms, primarily the mesozooplankton, which are the most important in the context of vertical export due to their facilitation of short food webs and the rapid conversion of smaller suspended

particles into larger sinking particles (Michaels & Silver, 1988). Studies of the mesozooplankton at BATS began in 1989, with the addition of 0-150 m daytime net tows to the recently established BATS programme. This protocol was enhanced in April 1994 to include both day and night tows between the surface and 200 m, and these continue to be carried out on a monthly basis to the present day (Madin *et al.*, 2001). In addition, samples generated from these tows are being used in a variety of complementary studies, including analysis of species composition and abundance, and assessing the correlation between sinking flux and salp abundance (D.K. Steinberg, pers. comm.). Other zooplankton studies at BATS have included a process study as part of the "ZOOSWAT" programme (Roman *et al.*, 1993, 1995), the quantification of various processes contributing to the active flux (Dam *et al.*, 1995; Steinberg *et al.*, 2000, 2002; Schnetzer & Steinberg, 2002a), the feeding of vertical migrants (Schnetzer & Steinberg, 2002b), the distribution and vertical migration of salps (Madin *et al.*, 1996), and the estimation of mesozooplankton production (Roman *et al.*, 2002).

According to Moore (1950), the main scattering layer around Bermuda lies between 400 and 600 m during the day, and the deep layers may reach down to 1000 m or more. What does not appear to have been described in the literature is what happens to these layers at night. However, results from the BATS programme have demonstrated the strong influence of interzonal DVM behaviour in this region (Madin *et al.*, 2001, and see Figure 5.4). These results have shown that biomass in the top 200 m generally increases by 1.5 to 2.5 times at night (max. = 3.4), although there have been several occasions (6 out of 56) on which nighttime decreases in biomass have been seen. In terms of dry weight, the nighttime biomass in the upper 200 m at BATS has shown a mean monthly increase of 251 mg DW m^{-2} d^{-1}, with a maximum of 1732 mg DW m^{-2} d^{-1} recorded in March 1998 (cf. mean 394 mg DW m^{-2} d^{-1} for the HOT site off Hawaii: Al-

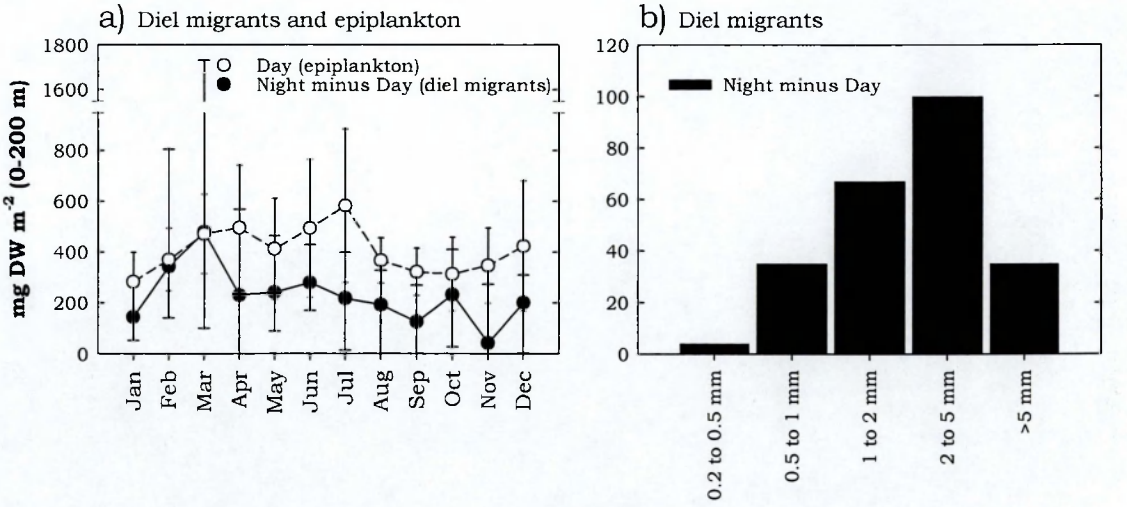

Figure 5.4 The zooplankton dry-weight (DW) biomass in the surface 200 m at BATS from tows made with a 1 m^2, 200 μm mesh net between April 1994 and December 1998. a) Mean monthly biomass (±1SD) of interzonal migrants (night-day difference in biomass) and epiplankton (day biomass). b) The mean contribution of each size fraction to the interzonal migrant biomass. Graphs reproduced from data provided in Madin *et al.* (2001).

Mutairi & Landry, 2001). If we were to apply this mean value to the whole of the Sargasso Sea, which covers an area of ~5 million km^2, this would indicate that ~0.001 Pg (dry weight) of zooplankton (~ 1 million tonnes) migrate into the upper 200 m every night. Analysis of the various size fractions has revealed that these migrations are mainly due to larger organisms, especially those in the 2-5 mm class (Madin *et al.*, 2001). Other studies (e.g. Roman *et al.*, 1993; Dam *et al.*, 1995) are generally in agreement with this.

Steinberg *et al.* (2000) and Madin *et al.* (2001) listed the dominant species of interzonal zooplankton vertical migrators at BATS. These included the copepods *Pleuromamma xiphias*, *P. abdominalis*, *P. gracilis* and *Euchirella messinensis*, the krill *Thysanopoda aequalis*, *Nematobrachion flexipes* and *Euphausia hemigibba*, the hyperiid amphipods *Anchylomera blossevillei* and *Scina* spp., the sergestid shrimps *Sergia splendens*, *Sergestes atlanticus* and *S. vigilax* and the alciopid worm *Naiades* sp..

Steinberg *et al.* (2000) also showed that *Pleuromamma* spp. and *T. aequalis* alone made up a significant percentage of the zooplankton biomass in the surface layer at night (mean = 23 %, range = 4-70 %). Ongoing analysis of the preserved BATS time-series samples, however, is revealing a more diverse krill community than at first thought, including at least two species of *Euphausia*, one species of *Nematobrachion*, two species of *Nematoscelis* and five species of *Stylocheiron* (D.K. Steinberg & S.E. Wilson, pers. comm.).

The bulk of zooplankton studies in the Sargasso Sea have therefore concentrated on the surface 500 m, with the occasional series of depth-discrete tows down to 2000 m. However, there has been no published information to date on the timing and amplitude of interzonal migrations, on the numbers involved, on seasonal changes in behaviour or on the proximate and ultimate causes of individual and population movements for any of the above species in this region (except for the study of Buskey *et al.*, 1989). Given the increasing use of the BATS dataset for a variety of studies, and the widely recognised biogeochemical importance of the zooplankton within the open-ocean ecosystem (e.g. Longhurst & Harrison, 1988, 1989), the quantification of these parameters at BATS represents both an interesting and timely focus for research efforts.

***Pleuromamma* spp.**

Pleuromamma xiphias and *P. abdominalis* are important contributors to the migrating community at BATS, and *P. xiphias* was collected for analysis during the present study. The genus *Pleuromamma* (Giesbrecht & Schmeil, 1898) has been reviewed by Steuer (1932), and consists of nine species: *abdominalis*, *borealis*, *indica*, *scutullata*, *gracilis*, *piseki*, *quadrungulata*, *robusta* and *xiphias*. Members of this genus are characterised by a dark pigment spot, which is thought to be a luminous organ, on either the left or right

side of the cephalothorax (e.g. Blades-Eckelbarger & Youngbluth, 1988), from which the name "Pleuromamma" is derived (Pleuro, *side* + mamma, *breast*). *P. xiphias* (Giesbrecht, 1889) is mesopelagic, and has been found in tropical and subtropical parts of the Atlantic, Indian and Pacific Oceans. Adult females range in size (total length) from 3.5 to 5.9 mm, and adult males from 4.0 to 6.4 mm, making this the largest species in the genus. In the Sargasso Sea, where the smaller (2.4 to 4.4 mm) yet similar *P. abdominalis* is also abundant, *P. xiphias* may be further distinguished by the possession, in females, of a single small hook on the anterior proximal end of each of the antennae (*P. abdominalis* females have two).

P. xiphias is known to undertake significant DVM. By day, populations generally reside between 400 and 950 m or more, with the greatest concentrations typically around 450 to 550 m, while at night they are typically found between the surface and 950 m, with one or more concentration maxima between 75 and 250 m (e.g. Roe, 1972a, b; Buskey *et al.*, 1989; Wiebe *et al.*, 1992). Wiebe *et al.* (1992) calculated vertical swimming speeds of 36-39 mm s^{-1} in adult females, and 37.5-40 mm s^{-1} in adult males. Buskey *et al.* (1989) showed a peak sensitivity to light at 480 nm, and a peak negative phototaxis at a light level of 7.2×10^{11} photons m^{-2} s^{-1}. Evidence from mouthpart morphology, gut contents and laboratory experiments has demonstrated an omnivorous diet (e.g. Arashkevich, 1969; Itoh, 1970; Harding, 1974). Schnetzer & Steinberg (2002b) analysed the natural diet of *P. xiphias* at BATS, and showed that feeding habits agreed with the predictions from mouthpart morphology. They showed that the diet generally reflected the seasonal changes in the phytoplankton community. Herbivory, particularly on diatoms, was predominant from late winter through to summer, with more emphasis on carnivory from autumn to early winter. Prey items included protozoans, crustaceans, chaetognaths and cnidarians. In addition, detritivory was

important at most times of the year, with food items including marine snow from larvacean houses. *Pleuromamma* spp. may form part of the diet of a number of higher predators, including euphausiids (Mauchline, 1980) and mesopelagic fish (e.g. Uchikawa *et al.*, 2001), to which end they have developed a powerful escape response and the ability to produce bioluminescent discharges (e.g. Hartline *et al.*, 1999; Lenz *et al.*, 2000; Weatherby *et al.*, 2000).

Krill

The krill collected during this study were not identified to species level, and none were preserved for future analysis. In the light of recent findings (D.K. Steinberg & S.E. Wilson, pers. comm.), it is likely that these samples would have consisted of a number of species. The length range of the samples (6 to 16 mm) would indicate that any of these species could potentially have been included. However, considerations of the catching properties of the net used, and the general abundance and size of species in previous BATS time-series tows at the same time of year (Figure 5.5), would suggest that the samples taken during this study were mainly composed of *Thysanopoda aequalis* (12 to 22 mm), *Euphausia hemigibba* (9 to 16 mm) and *Euphausia brevis* (8 to 10 mm) (S.E. Wilson, pers. comm.). Each of these species are known to undertake significant DVM, particularly in the post-furcilia stages. Of these species, *T. aequalis* has received the most attention in the literature. Populations of *T. aequalis* have been found to reside between 300 and 800 m during the day, and between 100 and 400 m at night (e.g. Lewis, 1954; Brinton, 1967; Wiebe *et al.*, 1992; Gibbons *et al.*, 1999). Wiebe *et al.* (1992) estimated swimming speeds of around 53-54 mm s^{-1}, and they also noted that the time of arrival at the surface during this particular study was ~1 h ahead of that for *P. xiphias*.

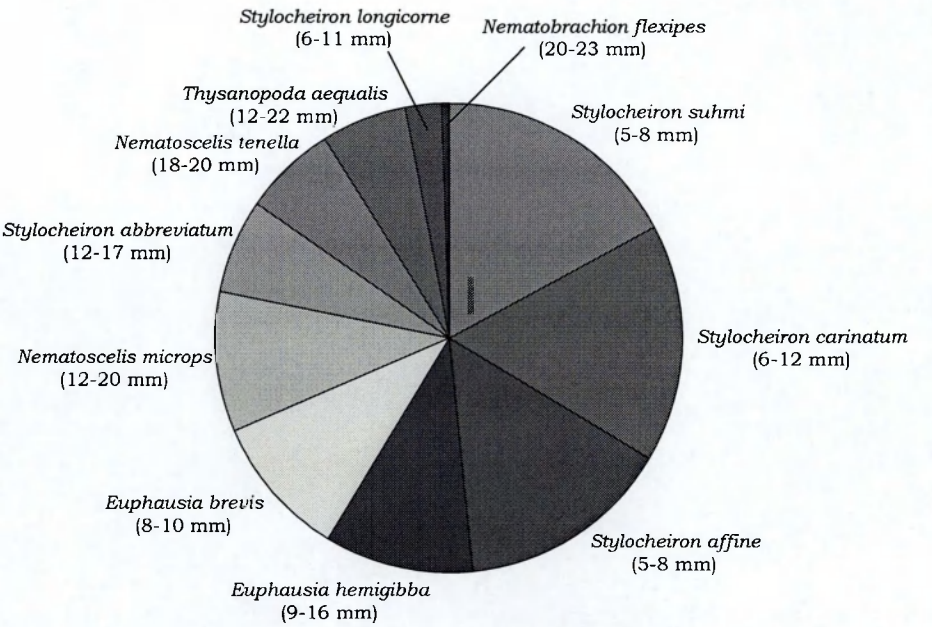

Figure 5.5 The mean composition of the krill community at BATS from net tows made in August 1996, September 1996, September 1997, October 1997, August 1998 and September 1998. Data courtesy of S.E. Wilson at VIMS. Numbers in brackets refer to the length range of adults (from Baker *et al.*, 1990).

The mouthparts of *Thysanopoda* spp. are heavily setose, and therefore suitable for filter feeding (Mauchline & Fisher, 1969). *T. aequalis* is both predatory and omnivorous, feeding on detritus, "algae", diatoms, dinoflagellates, tintinnids, radiolaria and foraminifera by filter feeding, and chaetognaths and "crustacea" by "encounter feeding" (Mauchline & Fisher, 1969; Casanova, 1974; Roger, 1974, 1975). Roger (1975) stated that species of this genus tend to feed continuously, although he had previously shown evidence of a certain degree of diel rhythmicity in the feeding of *T. aequalis* (Roger, 1973): the most active feeding occurred in the surface layer at night (20:00-06:00 h), a secondary period of slightly reduced feeding occurred between 400 and 500 m from noon to dusk (12:00-20:00 h), and generally low levels of feeding occurred during the rest of the day. Furthermore, he suggested that the stomach is filled more than once during the night, and that the diet may change from herbivorous at the surface to carnivorous at depth. As with *P. xiphias*, Schnetzer & Steinberg (2002b)

showed that the natural diet of *T. aequalis* at BATS agreed well with predictions from mouthpart morphology, and that a variety of phytoplankton, zooplankton and detritus were utilised as food. The literature provides equivocal evidence of the link between diel feeding rhythmicity and DVM behaviour (Ponomareva, 1971; Roger, 1975; Hu, 1978). There appears to be very little information regarding the feeding of *E. hemigibba* and *E. brevis*. *T. aequalis*, and presumably *E. hemigibba* and *E. brevis* as well, may form part of the diet of a range of predators, including epi-, meso- and bathypelagic fish, sergestid shrimps, deep-sea copepods, giant ostracods, lobate ctenophores, pelagic decapods, birds and other krill (see Mauchline, 1980, and references therein). Since *T. aequalis* does not tend to form large aggregations, it is less likely to be preyed upon by whales, seals and squid (Mauchline, 1980).

5.2 Materials and methods

5.2.1 Sampling sites and schedule

Sampling was carried out in the Sargasso Sea near Bermuda at two well known biogeochemical time-series study sites: Hydrostation S (32.2 °N, 64.5 °W), which lies 26 km south-east of Bermuda in 2500-3000 m of water, and the US JGOFS Bermuda Atlantic Time-series Study (BATS) site (31.7 °N, 64.2 °W), which lies 82 km south-east of Bermuda in ~4680 m of water (Figure 5.1). These sites were visited on six separate occasions during the summer of 2000 (Table 5.1) aboard the R/V "Weatherbird II", a 35 m University-National Oceanographic Laboratory System (UNOLS) research vessel operated and maintained by the Bermuda Biological Station for Research (BBSR). The 1 d cruises to Hydrostation S (HS916, HS918, HS919) were used to develop the methodology, while the 2-5 d cruises to the BATS site (BATS143, BATS144, BVAL29) were used to investigate the active flux.

Cruise number (site)	Sampling dates in 2000
HS916 (Hydrostation S)	July 24
BATS143 (BATS site)	August 7 – 11
HS918 (Hydrostation S)	August 21
HS919 (Hydrostation S)	September 5
BATS144 (BATS site)	September 11 – 15
BVAL29 (BATS site)	September 28 – 29

Table 5.1 Sampling dates of the time-series cruises conducted in the Sargasso Sea in 2000, of which the present study was a part.

5.2.2 The BATS programme

The zooplankton collections carried out for this study were undertaken during several of the regular time-series cruises at Hydrostation S and BATS. A variety of ancillary data from these cruises was kindly made available ahead of their public release time to support this study. The following is a synopsis of sampling that takes place on a typical cruise, as described in the BATS methodology manual (available on-line at URL: http://www.bbsr.edu) and Steinberg *et al.* (2001).

Core measurements at BATS

The BATS cruises consist of a single 4-5 d cruise at monthly intervals. The core measurements (Table 5.2) are made from two CTD package deployments ("hydrocasts"), one dawn to dusk *in situ* measurement of integrated primary production and a 3 d sediment trap deployment (Particle Interceptor Traps, PITS). Firstly, the sediment traps are deployed 5 nautical miles south of the BATS site, where they are equipped with a strobe, radio beacon and an ARGOS satellite transmitter, and left to drift for 3 d. Of the two hydrocasts, a deep cast to 4200 m is usually made first, with the water bottles being fired at 100 m intervals from 300 to 1400 m, at 200 m intervals from

Parameter	Depth (m)	Technique/Instrument
Continuous electronic measurements		
Temperature	0-4200	Thermistor on SeaBird SBE-911 *plus* CTD
Salinity	0-4200	Conductivity sensor on SeaBird SBE-911 *plus* CTD
Depth	0-4200	Digiquartz pressure sensor on SeaBird SBE-911 *plus* CTD
Dissolved oxygen	0-4200	SeaBird Polargraphic Oxygen Electrode
Beam attenuation[a]	0-200	SeaTech 25cm Transmissometer
Fluorescence	0-500	Chelsea MkIII Aquatracka Fluorometer
PAR[a]	0-200	Biospherical Scalar Irradiance Sensor, 400-700nm
Discrete measurements from Niskin bottles on CTD		
Salinity	0-4200	Conductivity on Guildline Autosal 8400A
Oxygen	0-4200	Winkler Titration, automated UV endpoint detection
Total CO_2	0-500	Automated coulometric analysis
Alkalinity	0-500	High precision titration
Nitrate	0-4200	CFA colorometric with Technicon AA
Nitrite	0-4200	CFA colorometric with Technicon AA
Phosphate	0-4200	CFA colorometric with Technicon AA
Silicate	0-4200	CFA colorometric with Technicon AA
Dissolved organic carbon	0-4200	High-temperature combustion
Dissolved organic nitrogen	0-4200	UV oxidation
Particulate organic carbon	0-4200	High-temperature combustion, CHN analyser
Particulate organic nitrogen	0-4200	High-temperature combustion, CHN analyser
Particulate silica	0-4200	Chemical digestion, colorometric analysis
Fluorometric chlorophyll *a*	0-250	Acetone extraction, Turner fluorometer
Phytoplankton pigments	0-250	HPLC, resolves 19 pigments
Bacteria	0-3000	DAPI stained, fluorescence microscopy
Rate measurements		
Primary production	0-140	Trace-metal clean, *in situ* incubation, ^{14}C uptake
Bacterial activity	0-1000	(^{3}H-methyl) thymidine incorporation
Particle fluxes	150, 200, 300	Free-drifting cylindrical trap (MultiPITS)
Mass flux		Gravimetric analysis
Total carbon flux		Manual swimmer removal, CHN analysis
Organic carbon flux		Manual swimmer removal, acidification, CHN analysis
Organic nitrogen flux		Manual swimmer removal, CHN analysis

[a]Currently measured on BATS cruises as part of the Bermuda Bio-optical Program (BBOP)

Table 5.2 The core measurements made monthly in the Sargasso Sea as part of the US JGOFS Bermuda Atlantic Time-series Study (BATS) (reproduction of Table 1 in Steinberg *et al.*, 2001).

1400 to 2600 m, and then at 3000 (duplicates), 3400, 3800, 4000 and 4200 m. During the second shallow cast to 250 m, two bottles are fired at 0, 10, 20, 40, 60, 80, 100, 120, 140, 160, 200 and 250 m. Water sampling takes place immediately after the CTD is brought on board according to the following protocol:

1. *Deep cast.* Samples are drawn from the OTE bottles in the following order: oxygen, DOC and DON, salinity, nutrients. Samples for POC, PON and particulate silicate (PSi) are taken from the top eight depths. Samples for bacterial enumeration are drawn at 3000 and 4000 m.
2. *Shallow cast.* Samples for oxygen, total CO_2 (C_T), alkalinity, DOC and DON, salinity and nutrients are drawn from one set of bottles at all depths. The replicate depths are used for POC, PON, PSi, fluorometric chlorophyll, HPLC pigment determination and bacterial enumeration.

The primary production array is deployed an hour before dawn on the second day, following the pre-dawn collection of water samples. The ship follows the array during its 12-15 h deployment, occasionally shuttling back to the sediment trap location for a visual inspection. At dusk, the array is recovered and processed immediately. The last operation before returning to shore is generally the recovery of the sediment traps.

Zooplankton sampling

While not strictly a part of the core programme, zooplankton have been collected at BATS since April 1994 using a 1 m^2 rectangular 202 μm-mesh net (Madin *et al.*, 2001). Temperature and depth over time have been recorded since June 1995 with a Vemco Minilog time-depth recorder (TDR), and the volume of water filtered measured with a General Oceanics mechanical flowmeter suspended across the centre of the net mouth. Two replicate double-oblique tows lasting approximately 30 min are made during the day (between about 09:00 and 15:00 h) and at night (between about 20:00 and 02:00 h) on each BATS cruise. Tows are made through the mixed layer to a depth of approximately 200 m. All depth-integrated biomass data are normalised to a 200 m

depth by the formula:

$$\text{Biomass}\,(0 \rightarrow 200\text{m}) = \text{Biomass}\,[0 \rightarrow \text{Xm}] \times \frac{200\text{m}}{\text{Xm}}$$

Equation 5.1

where X is the actual depth of the tow. Samples from the tows are split immediately on board, one half being used to make a silhouette photograph followed by preservation in 5 % buffered formalin, the other being wet-sieved through a series of five nested sieves. The sieve sizes are 0.2, 0.5, 1, 2 and 5 mm. The individual zooplankton fractions are transferred to tared discs of 0.2 mm Nitex netting and then frozen. Back ashore, the samples are thawed, blotted on absorbent paper, and weighed (wet weight) using a Sartorius analytical balance. Following oven-drying at 60 °C for 24 h, they are weighed again (dry weight). Carbon and nitrogen weight has been measured on a Control Equipment Corporation (CEC) 240XA elemental analyser using subsamples of the replicate size-fractionated day and night tows from four cruises per year (January, April, July and October) from 1994 to 1998. Well mixed aliquots of the dry samples were carefully homogenised in a pestle and mortar before combustion in the analyser.

Acoustic measurements

The Bermuda Testbed Mooring (BTM) has been deployed near the BATS site since June 1994 (Dickey *et al.*, 2001), providing the oceanographic community with a deep-water platform for developing, testing, calibrating and intercomparing instruments which can obtain long-term data sets. With the kind permission of Tommy Dickey and colleagues at the Ocean Physics Lab (OPL) at the University of California, Santa Barbara (UCSB), it has been possible to access the acoustic backscatter data from the

Figure 5.6 A diagrammatic representation of the instrumentation present during deployment 12 (July 29th to November 6th 1999) of the Bermuda Testbed Mooring (BTM). Diagram obtained from the Ocean Physics Laboratory website (URL: http://www.opl.ucsb.edu/btm.html).

upward-looking 150 kHz acoustic Doppler current profiler (ADCP), situated at 203 m depth. Figure 5.6 shows the instrumentation present during a typical deployment (deployment #12, July to November 1999). Further information is available on the world-wide-web (URL: http://www.opl.ucsb.edu/btm.html).

5.2.3 WP-2 net tows

A 2 m-diameter, 500 μm-mesh WP-2 net (Unesco, 1968) with a large volume (5 litre) clear perspex cod-end (see Steinberg *et al.*, 2000) was used to collect *Pleuromamma xiphias* and krill at various times during their DVM cycle (Figure 5.7). When available, a Vemco Minilog TDR fastened to the net's metal ring allowed the tow profile to be recorded. Tows were undertaken primarily around dawn and dusk, since this is when

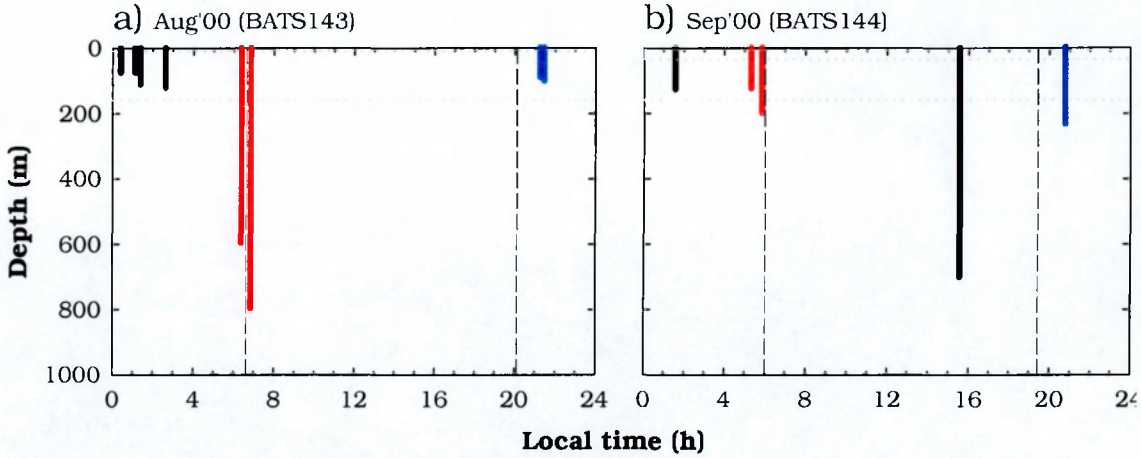

Figure 5.7 The time and depth of WP-2 net tows made at BATS during the present study for the collection of *P. xiphias* and krill. The red and blue lines represent 'dawn' and 'dusk' tows, respectively, and the thick black lines represent tows made at other times. The broken vertical lines show the timings of sunrise and sunset. The dotted horizontal lines show the extent of the pycnocline.

individuals were assumed to be performing their daily movements to and from the surface waters. Collections were also made at other times of day and night depending on the availability of time in the sampling schedule. The net was towed obliquely (at ~0.4 m s^{-1}) from a moving ship (speed through water ~1 kn) via the aft A-frame. The net was open for the whole tow. The depth of each tow was based on an assessment of where the migrators were thought to be at that time. This assessment was made on a trial and error basis, with a variety of tow depths being tried until the migrants were located. It had been found previously that no portion of the migrant community remained in the surface layer during the day (D.K. Steinberg, pers. comm.). Therefore it was deemed unnecessary to make depth-discrete tows, as the likelihood of contamination by shallower non-migrating portions of the population was low.

Sample processing

Once the net was aboard, the TDR was removed and downloaded, and re-activated if an immediate subsequent tow was being made. The cod-end was removed, taken into the

onboard laboratory, and poured gently into a number of shallow, rectangular white plastic trays. Using a glass pipette, healthy individuals of *Pleuromamma xiphias* and krill (the bulk of which were most likely to have been *Thysanopoda aequalis*, *Euphausia hemigibba* and *E. brevis*; see section 5.1.5) were removed to a mesh-bottomed container placed in a bowl of local surface seawater. The characteristic behaviour of *P. xiphias* made this species relatively easy to locate and pick out from the trays. After swimming around for a few seconds in the centre of the tray, individuals tended to congregate at the edges, where they rested, often near the surface, with their heads touching the tray sides. The laterally located dark pigment spot, which was visible to the naked eye, provided further confirmation of their identity. After 5-10 min, or when the supply of animals in the trays was exhausted if sooner, the specimens were processed via one of two methods:

(1) The onboard method

Only copepods were processed using this method. Prosome length measurements and gut fullness estimates were made onboard prior to freezing. Copepods that had been transferred into the glass bowl from the trays were pipetted into a 5 cm-diameter, 500 μm-mesh sieve, which was placed in a petri dish containing just enough filtered seawater to cover the bottom of the mesh. This kept the animals moist, while reducing backwash caused by the roll of the ship. Under a binocular microscope, *P. xiphias* was sorted into adult males and females, measured (prosome length) using an eyepiece graticule precise to ±0.04 or ±0.09 mm (depending on magnification), and an estimate of individual gut fullness made by eye, as gut contents were visible through the semi-transparent body wall (this estimate was based on the relative length of the gut contents, and expressed on a scale of 0 to 100 by 5 % increments). For consistency, these

estimates were carried out by myself throughout. Once measured, individuals were transferred into pre-weighed Elemental Microanalysis Ltd. tin capsules (8 mm × 5 mm) with fine forceps. Between one and three individuals of each stage were placed into a capsule. The capsules, held in labelled 96-well microtitre plates, were stored in the freezer at –20 °C. From cod-end to freezer, this method took <30 min.

Back at BBSR, the frozen samples were oven-dried at 60 °C for ~48 h. The open tin capsules were placed in the oven still in their microtitre plates with the lids removed. A layer of aluminium foil was laid loosely over the top of the samples to prevent the possibility of contamination from residues on the inside of the oven. After drying, the microtitre-plate lids were replaced, and the plates stored in a desiccator for at least a day to restore the samples to ambient temperature. Ideally the desiccator should also be in the same room as the balance to be used for weighing. These measures reduce the amount of atmospheric moisture absorbed by the dried samples, and so reduce the errors incurred during weighing (see section 7.3.2). The tin capsules were closed up using forceps, reweighed on either a Sartorius electrobalance precise to ±10 μg, or a CAHN Model 4400 electrobalance precise to ±0.5 μg, and placed into individual nickel sleeves ready for elemental analysis. These samples were run through a Control Equipment Corporation (CEC) 240XA elemental analyser, precise to ±0.01 μg, to yield their total carbon and nitrogen weights.

(2) The ashore method

In an effort to reduce or negate the need to use a binocular microscope while in often substantial open-ocean swells, and to speed up the sorting process to reduce stress on the animals, a second method was tried. This involved freezing the copepods and krill immediately after their removal from the white plastic trays, and making all

measurements ashore following thawing. Gently moving the mesh-bottomed container out of the glass bowl left the animals stranded on the mesh. A plastic wash-bottle containing filtered seawater helped to move animals on the mesh for ease of access. Using fine forceps, individuals were transferred into 6-well microtitre plates, covered with a thin layer of filtered seawater, and stored in the freezer at –20 °C. From cod-end to freezer, this method took <10 min.

Back at BBSR, the frozen samples were removed from the freezer and allowed to thaw in their microtitre plates at room temperature for a few minutes. Using fine forceps, individuals were transferred to a petri dish containing a small amount of filtered seawater. Under a binocular microscope, *P. xiphias* was sorted into adult males and females, measured for length (and, on latter cruises, greatest prosome width) and gut fullness as for the onboard method, and transferred into pre-weighed Elemental Microanalysis Ltd. tin capsules (8 mm × 5 mm). Krill, which were not sorted in any way, were measured for length (middle of the eye to the end of the telson) and also transferred into tin capsules. The drying, weighing and elemental analysis procedures were carried out as for the onboard-processed samples (see above).

5.2.4 Starvation experiments conducted on *P. xiphias* and krill

In August 2000 (BATS143) and September 2000 (BVAL29), *P. xiphias* adults and krill were collected at dawn (as described in section 5.2.3), when they were predicted to be at their daily maximum dry, carbon and nitrogen weight, and incubated in 0.45 μm-filtered local surface seawater. This was to follow the time-course of body-weight loss in a starved individual, thus providing a baseline for the measurements of individuals taken directly in the field. As before, the contents of the cod-end were poured into plastic trays, and healthy individuals of *P. xiphias* and krill gently pipetted out into a mesh-

bottomed container sitting in a glass bowl of surface seawater. Care was taken to avoid including other plankton and detritus in the pipette, and, when transferring into the glass bowl, the pipette tip was placed under the water before ejecting its contents to minimise stress and damage to the animal.

Animals in the glass bowl were pipetted into one of five separate 500 cm^3 mesh-bottomed perspex containers, each located in a 1-litre labelled screw top plastic jar containing filtered seawater. For a few hours prior to adding animals, the sealed jars were kept in a cool-box containing surface seawater to maintain an approximate environmental temperature, and this was monitored using a Vemco Minilog TDR. Both *P. xiphias* and krill were incubated in the same containers. Between 9 and 25 individuals of *P. xiphias* were added to each jar, and between 1 and 13 individual krill. In the September experiment, numbers were recorded as each animal was added with the aid of hand-held tap counters. Once all animals had been added, the jars were returned to the cool-box and left in the dark for the requisite amount of time. Some animals from the original net tow were frozen directly, as for the ashore method, to act as time zero (control).

Time-points were at approximately 0, 1, 2, 3, 9 and 14 hours. The emphasis was placed on sampling the first 3 h, since this was thought to be when the bulk of the body-weight loss would be occurring. At each time-point, one jar was removed from the cooler. After removal of the screw top, the mesh-bottomed container was gently lifted out of the jar, leaving the animals stranded on the mesh. These samples were measured for length, gut fullness and body weight as for the ashore method described in section 5.2.3.

5.3 Results

5.3.1 The physical environment: CTD data

Figure 5.8 shows the profiles (0-4000 m) of temperature, salinity and dissolved oxygen at BATS in August 2000 (BATS143) and September 2000 (BATS144). In August, the mixed layer was particularly shallow, extending to ~10 m, with a mean temperature of 28.8 °C. A seasonal thermocline (10-160 m) was present below this layer, through which the temperature dropped rapidly from 28.7 °C at 10 m to 19.2 °C at 160 m (-0.06 °C m^{-1}). The salinity maximum for the whole water column (36.7 psu) was found at 90 m, while a peak in dissolved oxygen (7.1 mg l^{-1})* was present at 42 m. Between 160 and 400 m, temperature changed very little (mean = 18.7 °C, mean change = -0.005 °C m^{-1}), and this is most likely to have been the STMW formed during winter mixing (see section 5.1.2). Salinity decreased gradually through this layer, to 36.5 psu at 400 m. Dissolved oxygen levels decreased steadily from the peak at 42 m to a minimum (4.8 mg l^{-1}) at 780 m. Temperature then changed rapidly through the permanent thermocline (400-1200 m), decreasing from 18.1 °C at 400 m to 5.0 °C at 1200 m (-0.02 °C m^{-1}). Similarly, a permanent halocline was evident from 400 to 1000 m, through which salinity decreased from 36.5 to 35.1 psu. From 1200 m, temperature in the deep water layer decreased gradually from 5.0 °C at 1200 m to 2.2 °C at 4274 m (-0.0009 °C m^{-1}), and salinity decreased gradually from 35.1 psu at 1000 m to 34.9 psu at 4274 m. Dissolved oxygen increased from the minimum (4.8 mg l^{-1}) at 780 m to a maximum (8.6 mg l^{-1}) at 2000 m. Below this depth, oxygen levels remained uniformly high, at ~8.5 mg

* Dissolved oxygen levels at BATS are nominally measured in units of μmol kg^{-1}. To facilitate comparison with the data presented for the Clyde Sea Area (see section 4.3.1), these values were converted to mg l^{-1} on the understanding that 1 mol dissolved oxygen weighs 32 g and 1 l of seawater weighs 1 kg.

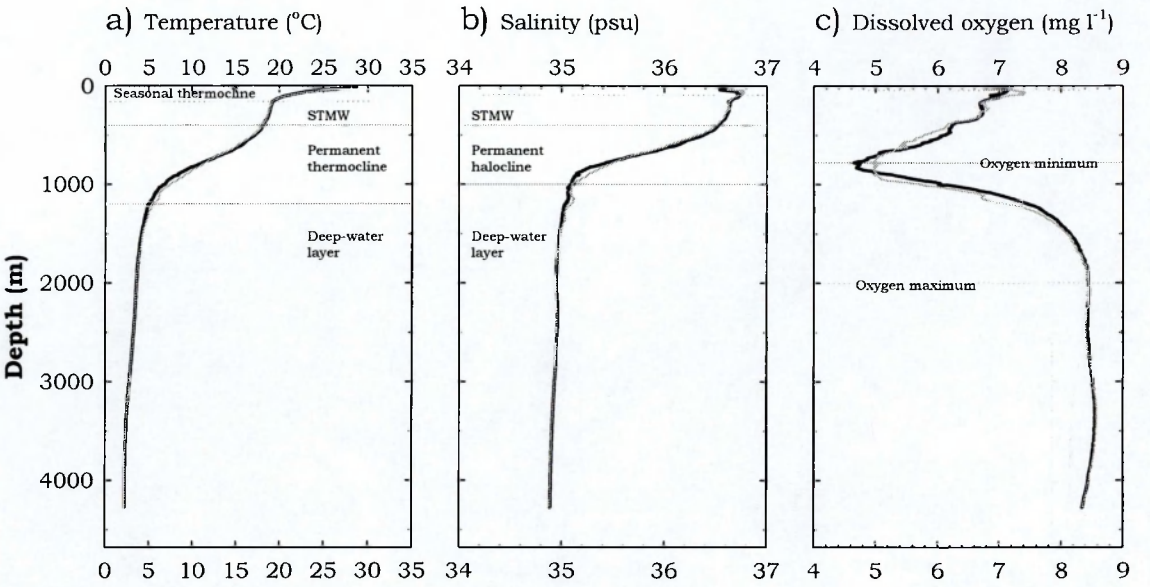

Figure 5.8 CTD-measured profiles of a) temperature, b) salinity, and c) dissolved oxygen at BATS in August 2000 (BATS143: black lines) and September 2000 (BATS144: grey lines). Individual readings were made at 1 m depth intervals. Processed data courtesy of P. Lethaby at BBSR.

l^{-1}. The profiles in September were similar, except for the surface mixed layer which was slightly cooler (mean = 28.1 °C) and extended deeper (to ~35 m).

5.3.2 Primary production: measurements of ^{14}C uptake and chlorophyll *a*

Figure 5.9a shows the depth profiles (0-140 m) of primary production (PP) at BATS in August 2000 (BATS143) and September 2000 (BATS144). PP peaked at 80 m in August, and 20 m in September, and dropped to low levels by 120 m in both months. These profiles did not correspond to the fluorometer-derived chlorophyll *a* levels, which increased rapidly from 0 to 100 m before decreasing to very low levels by 200 m (P. Lethaby, pers. comm.). Overall, PP in the top 140 m was twice as high in August as in September (Figure 5.9b). Interestingly, integrated PP (0-140 m) in August 2000 exceeded the maximum value recorded for this month during the period 1988-1998, while the September 2000 value was similar to its long-term mean.

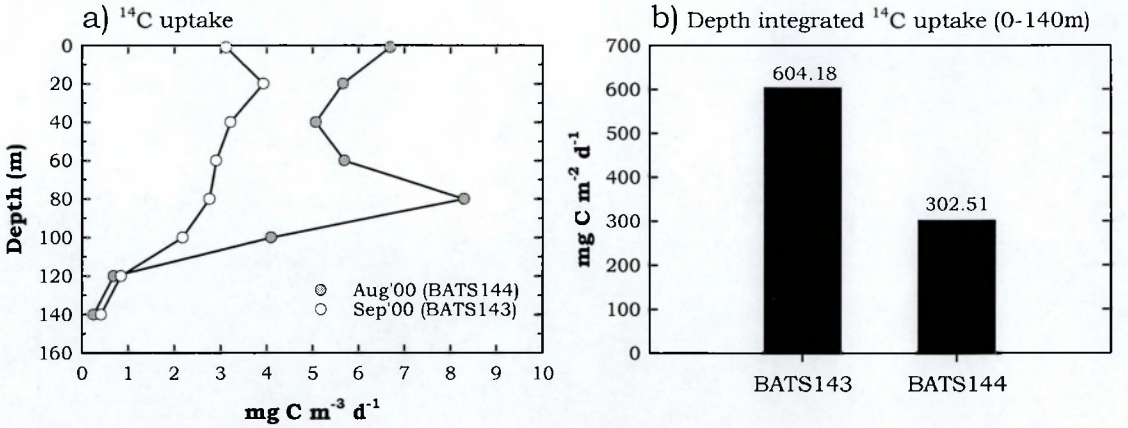

Figure 5.9 Primary production at BATS in August 2000 (BATS143) and September 2000 (BATS144), as measured from dawn-dusk *in situ* bottle incubations. a) Mean uptake of ^{14}C at 20 m depth intervals. b) Depth integrated ^{14}C uptake over the euphotic zone (0-140 m). Data courtesy of V. Lochhead at BBSR.

5.3.3 Sinking particle fluxes

Figure 5.10 shows the sinking particle fluxes (dry, carbon and nitrogen weight) at BATS at 150, 200 and 300 m in August 2000 (BATS143) and September 2000 (BATS144). Overall, fluxes were up to 34 % lower in September than in August (mean = 20 %), with the exception of PON at 150 m, which was actually 5 % higher. This was due, however, to a single replicate measurement being particularly high. In all cases, the flux of sinking material decreased with depth. In August, the sinking flux of POC at 150 m was 3.5 % of the depth-integrated (0-140 m) primary production (i.e. export ratio = 0.035). In September, the corresponding value had increased to 6 % (i.e. export ratio = 0.06).

5.3.4 Secondary production: mesozooplankton dynamics

Net-catch data

The biomass of the vertical migrant community: size-fractionated dry weight

Net tows made as part of the BATS time-series programme in August 2000 (BATS143) and September 2000 (BATS144) revealed nighttime increases in zooplankton dry

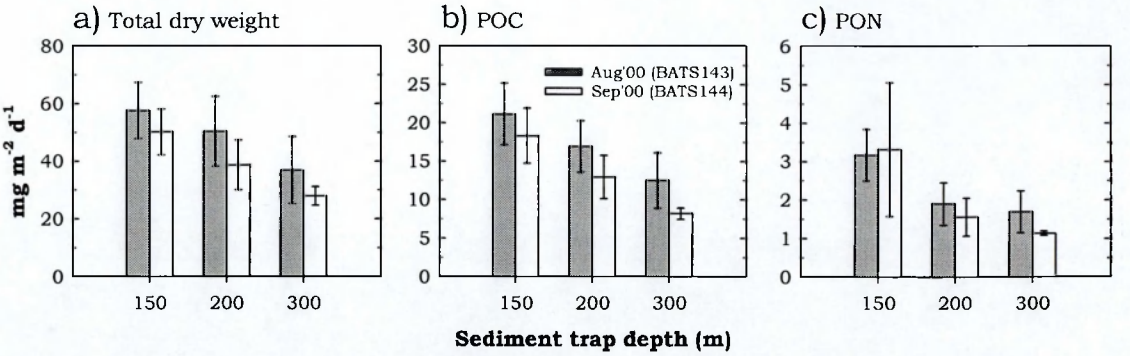

Figure 5.10 The sinking particle flux (mean ±1SD, n = 3) at BATS in August 2000 (BATS143) and September 2000 (BATS144), as measured from surface-tethered sediment traps at depths of 150, 200 and 300 m. a) Total dry mass, b) Particulate organic carbon (POC), and c) Particulate organic nitrogen (PON). Data courtesy of K. Kneely at BBSR.

weight in the top 200 m of 177 and 48 mg dry weight m^{-2}, respectively (Figure 5.11a). If one assumes that these increases were due to the arrival of zooplankton from below 200 m (as opposed to advected in from adjacent surface waters), this shows that interzonal DVM was a feature of the ecosystem in both months. In August, this inferred migrant community accounted for 30 % of the nighttime dry-weight biomass in the top 200 m. The greatest diel changes were seen in the 0.5-1, 1-2 and 2-5 mm size fractions, with nighttime increases of 54, 79 and 58 mg dry weight m^{-2}, respectively (Figure 5.11b). In September, the inferred migrant community accounted for 10 % of the nighttime dry-weight biomass in the top 200 m. The greatest diel changes were seen in the 0.2-0.5, 0.5-1 and 2-5 mm size fractions, with nighttime increases of 35, 24, and 26 mg dry weight m^{-2}, respectively. Of particular note was the nighttime decrease of 44 mg dry weight m^{-2} now seen in the dry-weight biomass of the 1-2 mm size fraction, and a nighttime increase of 8 mg dry weight m^{-2} in the >5 mm size fraction where no such increase was found in August. These observations show that the migrating biomass decreased from August to September, and that the size composition of the migrant community also changed noticeably.

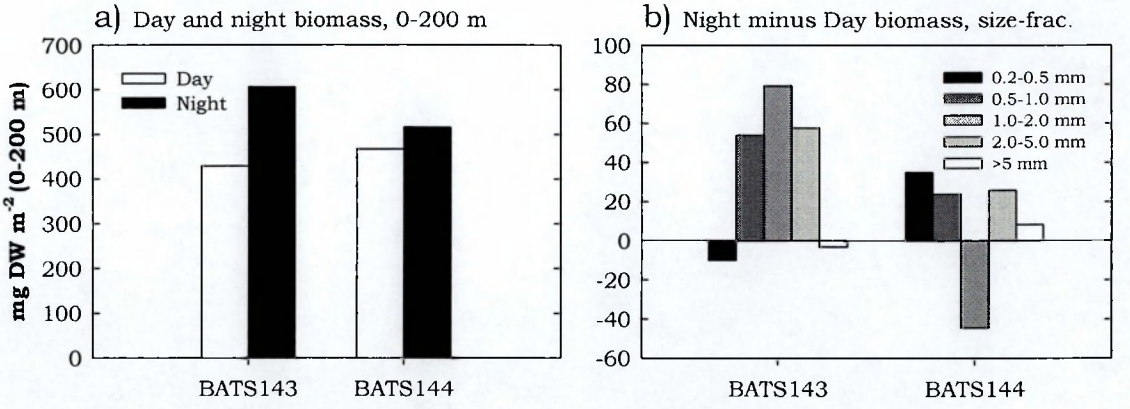

Figure 5.11 The zooplankton dry-weight (DW) biomass in the surface 200 m at BATS from tows made with a 1 m^2, 200 μm mesh net in August 2000 (BATS143) and September 2000 (BATS144). a) The mean monthly biomass (±1SD) of interzonal migrants (night/day difference in biomass) and epiplankton (day biomass). b) The mean contribution of each size fraction to the interzonal migrant biomass. A positive value indicates an increase in biomass in the top 200 m at night, and *vice versa*. Data courtesy of K. Cloutter at BBSR.

The numbers of P. xiphias and krill performing DVM

The numbers of individual *P. xiphias* adults and krill migrating to and from the top 200 m were estimated from the dry-weight biomass data by making a number of assumptions. Firstly, it was assumed that *P. xiphias* adults, with a prosome length ranging from 3.12 to 3.75 mm, and a prosome width ranging from 1.04 to 1.32 mm (measured during the present study), would have been found predominantly in the 2-5 mm size fraction, but that a very small proportion may have passed lengthways into the 1-2 mm fraction. Similarly, it was assumed that the krill collected during the present study, with lengths ranging from 6.6 to 16.6 mm (measured during the present study), would have been found in the >5 mm fraction, but that a potentially high proportion (of smaller individuals in particular) could have passed through lengthways into the 2-5 mm fraction. Based on this, Table 5.3 shows the percentage compositions (of the nighttime

	Percentage composition	
Size fraction	*P. xiphias* adults	Krill
1-2 mm	5	0
2-5 mm	35	45
>5 mm	0	40

Table 5.3 The estimated percentage contributions (of the nighttime increase in dry-weight biomass) by *Pleuromamma xiphias* adults (3.12-3.75 mm long, 1.04-1.32 mm wide) and krill (6.6-16.6 mm long) within the size fractions of the BATS-programme zooplankton net tows (see section 5.2.2 for a description of the sampling protocols).

increase in dry-weight biomass) that were assumed for each relevant size fraction. These data show that *P. xiphias* and krill combined would have made up 25 % of the interzonal migrant biomass in both months (i.e. [0.05×0.2]+[0.8×0.2]+[0.4×0.2] = 0.25).

From these estimates, the dry-weight biomass of migrating *P. xiphias* adults in August and September 2000 would have been 24.12 and 6.82 mg dry weight m^{-2} d^{-1}, respectively. With a mean individual dry weight of 563 µg in August 2000, and 547 µg in September 2000 (measured during the present study), the number of migrating individuals would therefore have been 42 and 12 ind. m^{-2} d^{-1}, respectively. Similarly, the dry-weight biomass of migrating krill in August and September 2000 would have been 24.67 and 14.94 mg dry weight m^{-2}, respectively. With a mean individual dry weight of 1338 µg in August 2000, and 1812 µg in September 2000 (measured during the present study), the number of migrating individuals would have been 18 and 8 ind. m^{-2} d^{-1}, respectively.

The timing and amplitude of DVM by P. xiphias and krill

Since depth-discrete net tows were not made during the present study, the timing and amplitude of DVM in *P. xiphias* adults and krill was estimated by a process of

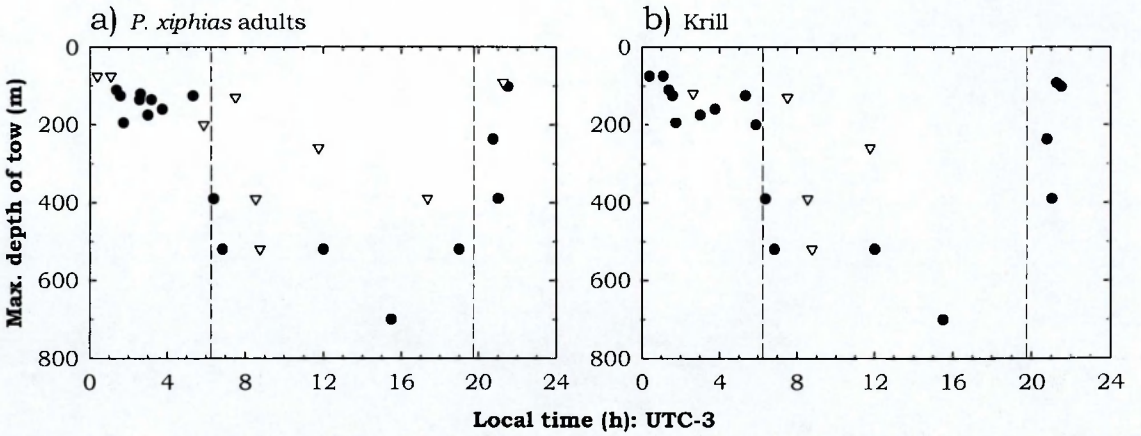

Figure 5.12 The presence (circles) or absence (triangles) of a) *Pleuromamma xiphias* and b) krill in depth-integrated WP-2 net tows made at Hydrostation S and BATS in July, August and September 2000. The vertical position of each symbol represents the maximum depth of the tow. The times of sunrise and sunset are indicated by dashed lines.

elimination from the presence or absence of individuals in the depth-integrated WP-2 tows (for times and depths, see Figure 5.7). Since a time/depth recorder (TDR) was not deployed on tows >200 m before September 2000, the exact depth of the net (d) in these instances was estimated from the amount of wire paid out (w) and the wire angle (a) according to the equation:

$$d = \text{cosine}\, a \times w$$

Equation 5.2

The wire angle was assumed to be 50° in all cases. This was calculated from a TDR-monitored net tow made in September 2000: with 800 m of wire paid out, the actual depth of the net was 520 m (i.e. cosine^{-1} (520/800) = 50°). Figure 5.12 shows the presence or absence of *P. xiphias* adults and krill in the depth-integrated net tows. In order to increase the temporal resolution for this exercise, tows made at Hydrostation S in July 2000 (HS916), August 2000 (HS918) and September 2000 (HS919) were also

included, based on the assumption that DVM would have been similar at these two sites. While the data strongly indicate that both species were undertaking NDVM (i.e. DVM cued by diel changes in light levels), there were also hints that there were differences in their vertical distributions at any one time. For example, *P. xiphias* adults were not found above 75 m at night, while krill were. It is also possible, from the absence of individuals in the top 120 m at 02:30 h and their subsequent presence again at 05:20 h, that krill performed 'midnight sinking' followed by a pre-dawn ascent. However, the possibility that horizontal patchiness and/or net avoidance was the cause of this observation cannot be discounted.

The dawn descent appeared to have been initiated earlier in *P. xiphias* than in krill. Where *P. xiphias* adults were present in the top 125 m at 05:20 h, they were absent from the top 200 m by 05:50 h, suggesting that the dawn descent took place around 05:30 h, or ~60 min before sunrise. Krill, on the other hand, were still present in the top 200 m at 05:50 h, and cannot be deemed absent from the top 130 m until 07:30 h at the earliest due to the lack of net tows between these times. During the day, both *P. xiphias* adults and krill migrants resided below 400 m at least, and quite possibly below 500 m on occasion. With an estimated swimming speed of 36-40 mm s^{-1} in *P. xiphias* (Wiebe *et al.*, 1992), it would have taken between 80 and 90 min for individuals to make their descent from 200 to 400 m. It can be estimated, therefore, that *P. xiphias* first reached its daytime residence depth approximately 20 to 30 min after sunrise at the earliest. For krill migrants with an estimated swimming speed of 53-54 mm s^{-1} (based on *T. aequalis*: Wiebe *et al.*, 1992), it would have taken ~60 min for individuals to descend from 200 to 400 m. It is likely, therefore, that the krill did not reach their daytime residence depth until at least 60 min after sunrise. The timing of the dusk ascent is less clear. Certainly *P. xiphias* was still below 400 m at 17:20 h (~150 min before sunset),

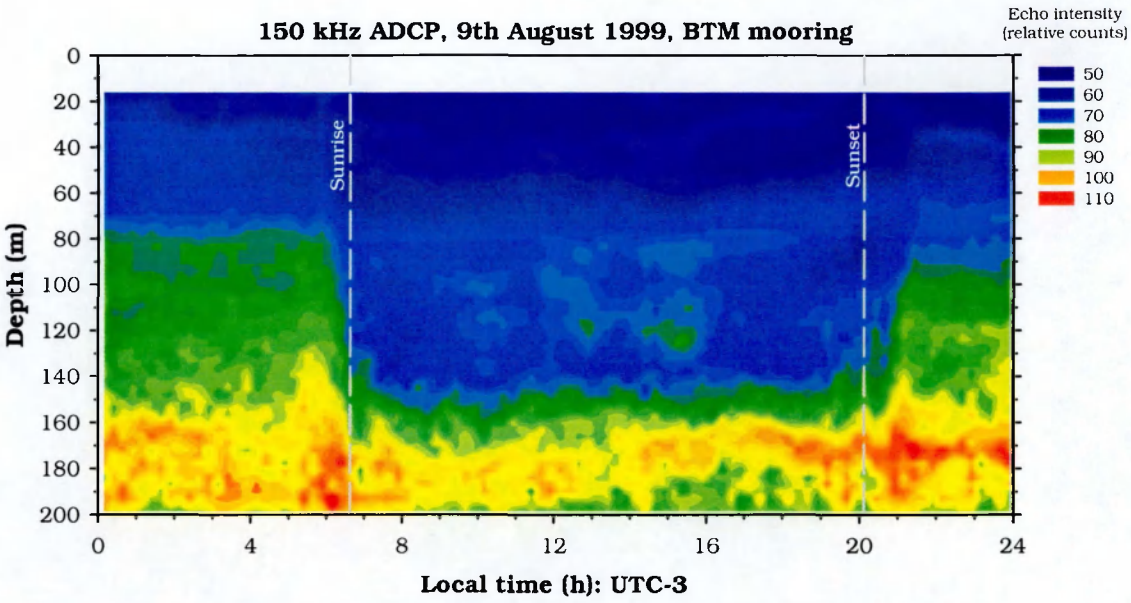

Figure 5.13 The echo intensity (relative counts) from backscattering particles in the water column at the BTM mooring-site on 9th August 1999, as measured by an upward-looking 150 kHz ADCP moored at 200 m. Data courtesy of T.D. Dickey at OPL, UCSB.

but was not confirmed in the top ~200 m until 20:45 h at the earliest (~60 min after sunset). Therefore, the earliest and latest times that *P. xiphias* could have first reached the top 200 m were between 70 min before, and 60 min after sunset, respectively. For krill, only the latest time of the first arrival in the top 200 m (i.e. ~60 min after sunset) could be confirmed.

Acoustic data

Figure 5.13 shows the acoustic backscatter on August 9th 1999 from an upward-looking 150 kHz ADCP at 200 m on the BTM mooring (near to BATS). The backscatter contours were generated by the same kriging algorithm used to create Figure 4.12 (see section 4.3.3). Only the raw backscatter data are shown (echo intensity, not converted to *Sv*), and only a single day has been chosen from the 8-year time-series record, as it is intended to provide merely a brief demonstration here of the biological information that

could be obtained from this particular dataset.

There was a noticeable diel change in the vertical distribution of backscattering particles within the top 200 m of the water column. A sound-scattering layer (SSL) was present from 140 m to at least 200 m throughout the diel cycle. Given that net tows to as deep as 500 m in the daytime during the present study (BATS143) did not catch any migrant species, migrant zooplankton can probably be discounted as the cause of this SSL during the day. There was relatively little backscatter in the top 140 m during the day. An increase in echo intensity between 90 and 140 m less than an hour after sunset indicated that backscattering particles had moved into this region from below. The shallowest depth limit of 80 to 100 m by the nighttime SSL is roughly coincidental with the shallowest limit of migrating *P. xiphias* at BATS estimated from net tows (Figure 5.12a). The increased level of backscatter in the 80-140 m depth interval decreased to its low daytime levels approximately 30 min before sunrise. This overall pattern is characteristic of zooplankton DVM reported in this region from net tows, suggesting that this dataset will be useful in studies of DVM behaviour at BATS.

Biometric measurements of P. xiphias and krill

Onboard vs. shore-based sample processing

P. xiphias adult males and females collected in July 2000 (HS916) and August 2000 (HS918) were processed via either the 'onboard method' or the 'ashore method' (see section 5.2.3) to investigate the effects of these different processing methods on the measurements of prosome length, gut fullness, and dry, carbon and nitrogen weight. The effect on prosome length was equivocal. In two out of four comparisons, there was no significant difference (ANOVA: $P > 0.05$), while in the remaining two cases, shore-processed individuals were found to be ~3 % longer (ANOVA: $P \leq 0.05$). Despite the

Cruise	No. of net tows	Taxon/ stage	Individuals		Samples			
			L values	GF values	Total no.	Ind. per sample	DW values	C&N Values
BATS143	5	*P.xiphias* m	61	60	30	1-3	30	30
		P.xiphias f	102	100	43	1-5	43	33
		Krill	77	0	77	1	77	22
BATS144	5	*P.xiphias* m	64	64	64	1	64	13
		P.xiphias f	132	132	132	1	132	29
		Krill	67	0	67	1	58	22
			503	**356**	**413**	**1-5**	**404**	**149**

Table 5.4 The number of *Pleuromamma xiphias* (adult males and females) and krill collected from WP-2 net tows at BATS and measured for length (L: prosome length in copepods, eye-telson length in krill), gut fullness (GF), dry weight (DW) and carbon and nitrogen weight (C & N).

statistical significance, the small difference makes it likely that prosome length was unaffected by changes in processing methodology.

In all cases, gut fullness was higher (by 10 to 40 %) in shore-processed individuals. This difference was significant in three out of four cases (ANOVA: $P < 0.001$). It is therefore likely that less gut material was being lost during shore-based processing. Conversely, shore-processed samples were found to have a lower mean weight. Dry weight was lower by 5-15 %, although this difference was only significant (ANOVA: $P \leq 0.05$) in one out of four comparisons. Carbon and nitrogen weights were lower by 7-25 %, although this difference was only significant (ANOVA: $P \leq 0.05$) for females in July 2000. In view of these relatively large differences, it is likely that both carbon and nitrogen were being lost during the shore-based processing. The significant difference (ANOVA: $P < 0.001$) between the C:N (atoms) of onboard-processed samples (C:N = 3.97) and that of shore-processed samples (C:N = 4.15) also suggests that a proportionately greater amount of nitrogen was being lost via this method.

Parameter	Precision	Data accuracy (%)		
		P.xiphias/male	*P.xiphias*/fem.	Krill
Prosome length	± 0.09/±0.04 mm	± 1.1 – 2.7	± 1.1 – 2.8	± 0.5 – 1.4
Dry weight	± 10/±0.5 µg	± 0.1 – 2.6	± 0.1 – 3.3	± 0.1 – 2.5
Carbon & nitrogen weight	± 0.01 µg	± 0.1	± 0.1	± 0.1

Table 5.5 The precision and accuracy range of biometric measurements made on *Pleuromamma xiphias* (males and females) and krill collected at BATS in August 2000 (BATS143) and September 2000 (BATS144).

Sample size and data quality

Table 5.4 summarises the number of samples collected and the number of biometric measurements made during each sampling cruise. These samples were shore-processed in all cases. Table 5.5 shows the precision of the measuring instruments used, and the subsequent accuracy of the biometric data. By way of quality control, only those data values with an accuracy of ±5 % or less were deemed trustworthy enough for inclusion in further analyses (see Equation 2.2). The combination of relatively high instrument precision and the large size of individuals and samples meant that all data were sufficiently accurate.

Relationships between parameters: investigating the ecology of individuals

Figure 5.14 shows that the body weight (dry, carbon or nitrogen weight) of *P. xiphias* and krill increased exponentially with increases in body length (i.e. $Y = aX^b$). The log transformation of the X and Y values therefore allowed this relationship to be described in linear terms (i.e. $\log Y = \log a + b \log X$). For *P. xiphias*, when data from August and September 2000 were combined, the log length/log weight relationships were relatively weak (r^2 ~0.3 in all cases), although they were statistically significant (ANOVA: P

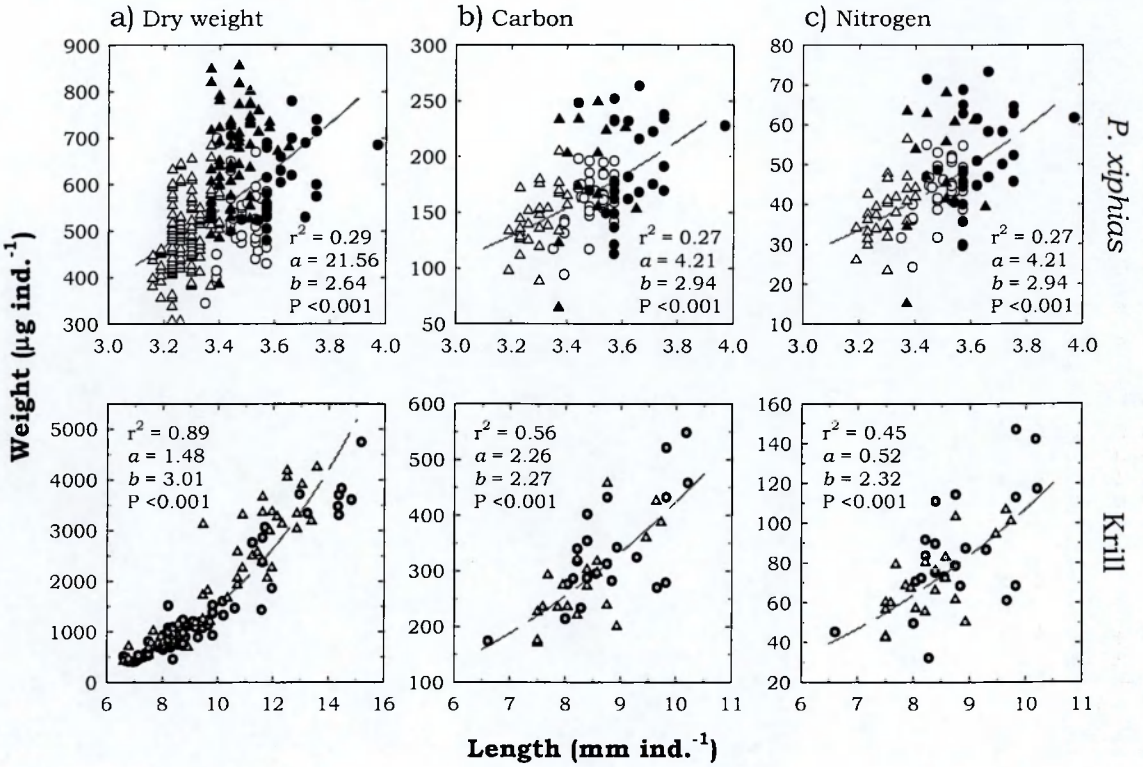

Figure 5.14 The relationship between the mean individual prosome length of *Pleuromamma xiphias* (upper graphs) and eye-telson length of krill (lower graphs) in different samples (n = 1-4 individuals per sample) and the mean individual body weight, in terms of a) dry weight, b) carbon weight, and c) nitrogen weight. The lines of best fit ($Y = aX^b$) and their regression parameters are shown on each graph. Symbol colours define the taxon and/or developmental stage: *P. xiphias* adult males = black, *P. xiphias* adult females = white, krill = black with white dot. Symbol shapes define the sampling date: Aug'00 (BATS143) = circles, Sep'00 (BATS144) = triangles.

<0.001 in all cases). That is to say, changes in prosome length explained only ~30 % of the changes in body weight. For krill, when data from August and September 2000 were combined, the log length/log weight relationships were relatively strong (r^2 = 0.45 to 0.89) and statistically significant (ANOVA: P <0.001 in all cases). That is to say, changes in body length explained between 45 and 89 % of the changes in body weight. In the case of *P. xiphias*, the length/weight relationship was highly variable, implying that the samples collected during each cruise consisted of individuals with different body conditions. In the case of krill, the length/weight relationship was much more

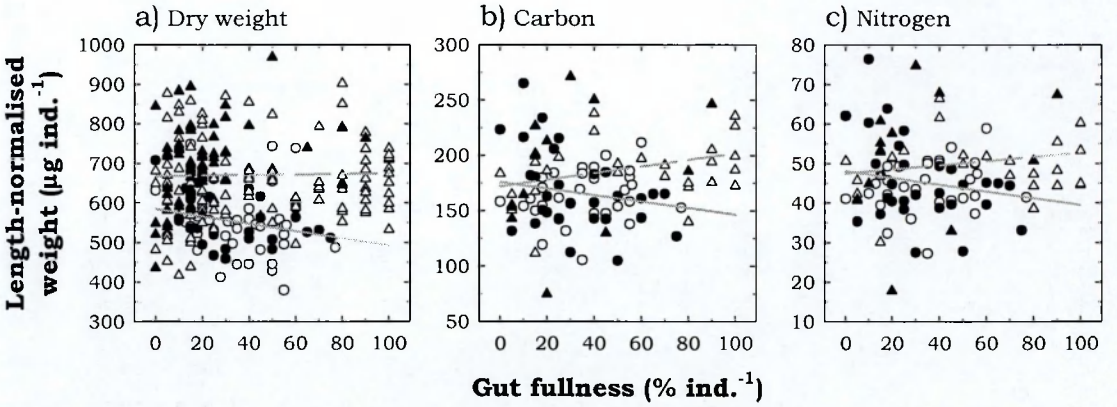

Figure 5.15 The relationship between the mean individual gut fullness of *Pleuromamma xiphias* in different samples (n = 1-4 individuals per sample) and the mean length-normalised (3.5 mm) individual body weight, in terms of a) dry weight, b) carbon weight, and c) nitrogen weight. The lines of best fit ($Y = a + bX$) are shown on each graph (bold lines = BATS143, broken lines = BAT144). See Figure 5.14 for an explanation of the symbols.

uniform, implying that the body condition of all individuals was similar.

Figure 5.15 shows that the body weight (dry, carbon or nitrogen weight) of size-normalised (3.5 mm-long) *P. xiphias* adults did not increase consistently with increases in gut fullness. Simple (least squares) linear regression analysis of gut fullness (X_i values) versus body weight (Y_i values) showed that the slope of each line, b, did not differ significantly from zero at the $\alpha = 0.05$ level (b = -0.92 to 0.29; ANOVA: P = 0.07 to 0.8). This implies that the gut contents of *P. xiphias* were not significant in terms of the overall mass of the individual, and therefore that defaecation would not represent a significant avenue for carbon and nitrogen loss in this species.

Figure 5.16 shows that the carbon and nitrogen weight of both *P. xiphias* and krill increased linearly with increases in dry weight (i.e. $Y = a + bX$, $b > 0$). Furthermore, these positive relationships were relatively strong ($r^2 > 0.6$) and highly significant (ANOVA: P <0.001 in all cases), suggesting that dry weight might represent a rough proxy for both carbon and nitrogen weight. Therefore, where dry weight but not carbon

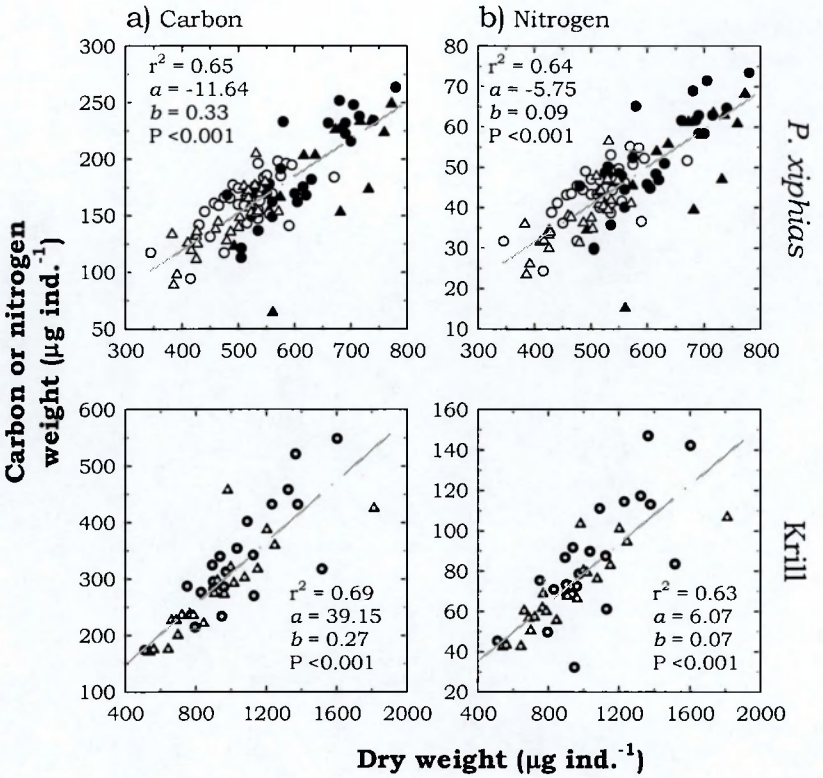

Figure 5.16 The relationship between the mean individual dry weight of *Pleuromamma xiphias* (upper graphs) and krill (lower graphs) in different samples (n = 1-4 individuals per sample) and the mean individual elemental composition, in terms of a) carbon weight, and b) nitrogen weight. The lines of best fit ($Y = a + bX$) and their regression parameters are shown on each graph. See Figure 5.14 for an explanation of the symbols.

or nitrogen weight measurements were made on a sample or individual (August 2000: 10 out of 73 *P. xiphias* samples, 52 out of 72 krill individuals; September 2000: 154 out of 196 *P. xiphias* samples, 36 out of 58 krill individuals), these relationships were applied to predict the carbon and nitrogen weight, and therefore increase the sample size for more robust statistical analysis.

Figure 5.17 shows that the nitrogen weight of both *P. xiphias* and krill increased linearly with increases in carbon weight (i.e. $Y = a + bX$, $b>0$). The C:N (atoms) of all individuals was found to be highly uniform (mean±1SD: *P. xiphias* = 4.35±0.16; krill = 4.68±0.63), implying that the C:N body condition of all individuals was very similar.

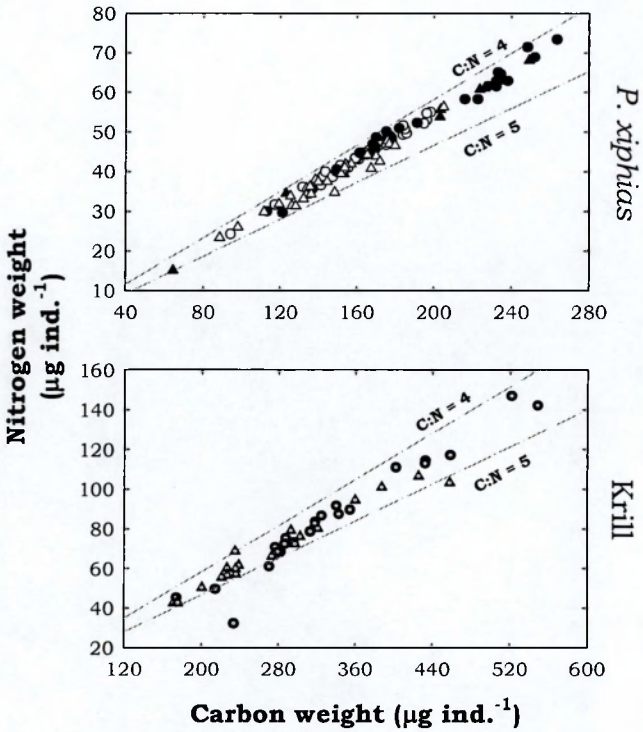

Figure 5.17 The relationship between the mean individual carbon weight of *Pleuromamma xiphias* (upper graph) and krill (lower graph) in different samples (n = 1-4 individuals per sample) and the mean individual nitrogen weight. Lines show the C:N (atoms) lines of equivalence. See Figure 5.14 for an explanation of the symbols.

Variability in the data

Table 5.6 shows the mean, standard deviation (SD) and coefficient of variation (V) of the various biometric measurements made on *P. xiphias* and krill collected at BATS during the period August to September 2000 (BATS143 and BATS144). The data were combined from each sampling date, but considered separately for *P. xiphias* males, *P. xiphias* females, and krill. For *P. xiphias*, the size-normalisation of the dry-, carbon- and nitrogen-weight measurements to an average body size (see section 2.3.6) did not appreciably reduce V when using either prosome length (3.5 mm) or prosome volume (3.5 mm^3)* as the measure of size. When using dry weight (580 µg), however, the

* Prosome volume was calculated from prosome length (L) and maximum width (W) measurements made on individuals collected during BATS144 using the formula for a prolate spheroid: $\pi LW^2/6$

Parameter	Analysis	Mean±1SD (*V* %) Males	Females	Krill
Length (mm)		3.53±0.11 (3.11)	3.37±0.12 (3.66)	9.86±2.37 (24.04)
Dry weight (μg)	Raw measure	644.9±96.4 (14.9)	501.8±78.0 (15.5)	1546.0±1092.4 (70.7)
	L-norm.	658.6±116.8 (17.7)	626.7±103.8 (16.6)	1553.6±369.8 (23.8)
	V-norm.	715.4±94.1 (13.2)	707.2±66.6 (9.4)	-
Carbon weight (μg)	Raw measure	188.4±43.8 (23.3)	154.2±26.0 (16.9)	305.3±91.8 (30.1)
	L-norm.	174.0±43.6 (25.0)	173.4±26.8 (15.5)	428.2±80.0 (18.7)
	V-norm.	199.7±51.9 (26.0)	198.8±23.6 (11.9)	-
	DW-norm.	175.5±28.2 (16.1)	180.5±17.6 (9.8)	440.3±56.3 (12.8)
Nitrogen weight (μg)	Raw measure	51.2±12.4 (24.2)	41.2±7.5 (18.2)	77.5±25.8 (33.3)
	L-norm.	47.3±12.5 (26.4)	46.7±7.3 (15.5)	109.3±24.6 (22.5)
	V-norm.	54.2±14.6 (26.9)	53.9±6.4 (11.8)	-
	DW-norm.	47.6±8.3 (17.4)	48.6±5.2 (10.6)	113.7±17.6 (15.5)

Table 5.6 The variability of the biometric measurements made on *Pleuromamma xiphias* (males and females) and krill collected at BATS during the period August to September 2000 (BATS143 and BATS144 combined). "L-norm.", "V-norm." and "DW-norm" refer to those measurements which were standardised to a mean body length, volume and dry weight, respectively, using ANCOVA (*P. xiphias* = 3.5 mm long, 3.5 mm^3 volume, 580 μg dry weight: krill = 10 mm long, 1.5 mg dry weight).

variability in both the carbon and nitrogen data was more appreciably reduced. For krill, the size-normalisation of the dry-, carbon- and nitrogen-weight measurements to a mean eye-telson length of 10 mm appreciably reduced *V*, as one might expect given the fact that multiple size-classes were included. Normalisation to a mean dry weight (1.5 mg) reduced the variability in carbon and nitrogen weight even more.

These observations support the idea that differences in the size of individuals between samples represent a significant source of variability in the body-weight data. However, the low magnitude of the reductions in the variability of the *P. xiphias* data, despite the noticeable variability in the size of individuals, highlights the fact that the ability of the size-normalisation procedure to reduce size-related variability in the data

does depend upon the strength of the relationship between the measure of size and the variable to be normalised. The fact that dry weight exhibited a stronger relationship with both carbon and nitrogen weight than length or volume did mean that dry weight reduced the variability in the data more than either prosome length or volume in this instance.

The size-structure of the P. xiphias and krill populations: body length measurements

Figure 5.18 shows the prosome-length distributions of *P. xiphias* adults collected in July 2000 (HS916), August 2000 (BATS143 and HS918) and September 2000 (HS919 and BATS144). Adult females varied in length from 3.12 to 3.66 mm, with cruise-mean lengths ranging from 3.28 to 3.47 mm. Adult males varied in length from 3.21 to 3.75 mm, with cruise-mean lengths ranging from 3.39 to 3.61 mm. The length distributions of both males and females varied relatively widely and were non-normal in all cases (Kolmogorov-Smirnov: $P < 0.001$). Significant inter-cruise differences were found in the median lengths of both males and females (males, Kruskal-Wallis: $H_4 = 112.760$, $P < 0.001$; females, Kruskal-Wallis: $H_4 = 177.279$, $P < 0.001$), suggesting that there had been a genuine change in the size structure of the population over time. For the samples at BATS, these differences were due to the length-at-maturity becoming shorter as the year progressed (Dunn's: $P < 0.05$). Within the course of a single day, no significant differences were found in the median prosome length of either males or females collected at different times (Kruskal Wallis: $P > 0.05$ for both cruises at BATS). At Hydrostation S, the picture was more complex. Females did not appear to show any marked change in length from July to September, and this was confirmed statistically (Dunn's: $P > 0.05$), while males in fact increased marginally in size during this time (Dunn's: $P < 0.05$).

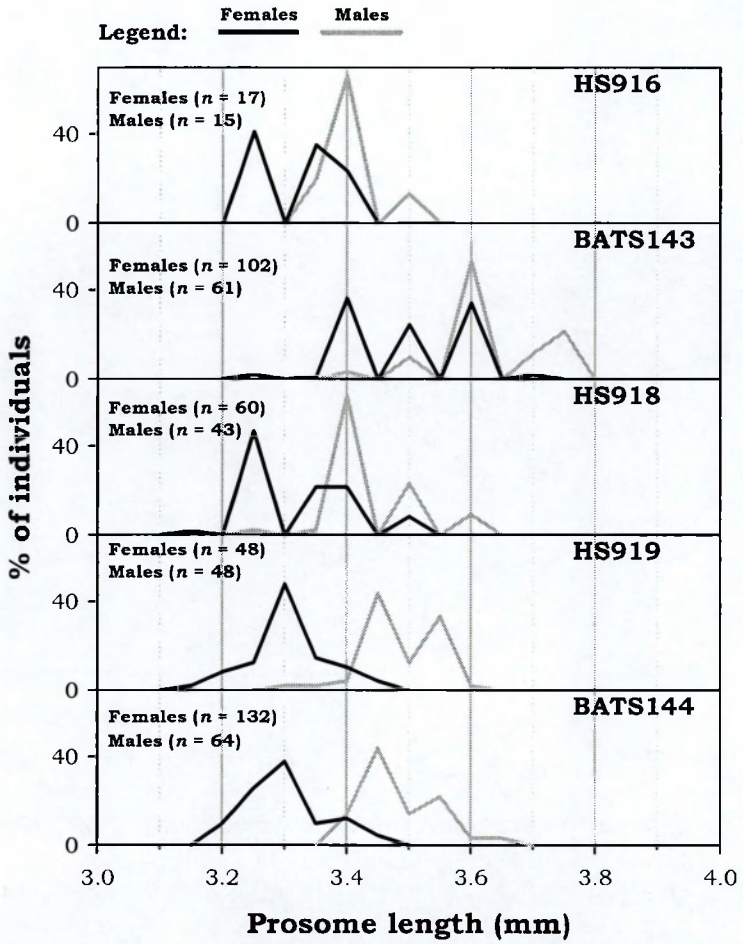

Figure 5.18 The prosome-length frequency (0.05 mm length classes) of *Pleuromamma xiphias* from Hydrostation S (HS) and BATS during the period July to September 2000, showing the distribution of adult males and adult females.

Given that specific developmental stages of krill were not targeted, it is apparent that the length data cannot be analysed in the same way as those from *P. xiphias*. It is therefore best simply to report the length distributions sampled during each cruise to give an idea of which species may have been included. While the mean size of sampled individuals showed a degree of variation between time-points in both August and September (8.6 to 11.5 mm), the range of lengths sampled at each time-point was similar (min: 6.6 to 8.0 mm; max: 12.9 to 16.6 mm). The krill collected during the present study at BATS consisted of individuals varying in length from 6.6 to 16.6 mm.

Diel patterns of feeding from gut fullness measurements

Temporal changes in the gut fullness of starved *P. xiphias* males and females were investigated via shipboard time-course experiments conducted at BATS in August 2000 (BATS143) and September 2000 (BVAL29) (incubation in 0.45 μm-filtered local surface seawater at 24 and 22.5 °C, respectively). The exact number of individuals added at the start of the experiment was counted in September but not in August. Figure 5.19 shows that the gut-fullness distribution of experimental individuals changed noticeably over the incubation periods. These differences, due to decreases in the median gut fullness over time, were found to be significant in both experiments (August, Kruskal-Wallis: $H_5 = 62.586$, $P < 0.001$; September, Kruskal-Wallis: $H_5 = 57.103$, $P < 0.001$). Furthermore, while the initial population (i.e. time zero) appeared to

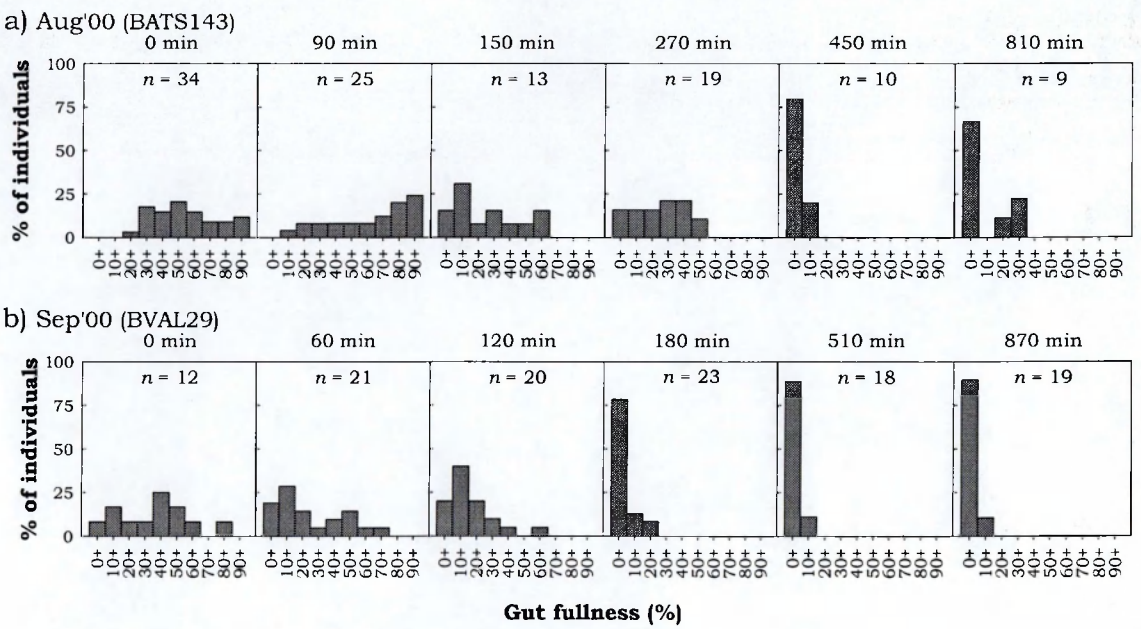

Figure 5.19 The gut-fullness distribution of *Pleuromamma xiphias* (adult males and females combined) collected at dawn at BATS in a) August 2000 (BATS143), and b) September 2000 (BVAL29) and maintained for varying lengths of time in the absence of food (incubation in 0.45 μm-filtered local surface seawater at 24 and 22.5 °C, respectively).

have been more well-fed in August (August: median = 50 %; September: median = 35 %), the time-course of relative gut evacuation was similar in both cases. After 90-120 min, the relatively small change in the population's median gut fullness (0-25 % reduction since time zero) was not significant (Dunn's: $P > 0.05$), and some individuals were still up to 100 % full. After 150-180 min, the larger change in the population's median gut fullness (30-35 % reduction since time zero) was significant (Dunn's: $P < 0.05$), and individuals ranged from 5 to 60 % full. Beyond 150-180 min, gut fullness continued to decrease, but at a low rate. After 250 min, there was still no evidence that complete gut evacuation had occurred, and individuals ranged from 5 to 50 % full. In fact, completely empty individuals were not found until the 450 and 510 min time-points in August and September respectively. This meant that complete evacuation of the guts by at least some of the experimental individuals had taken between 250 and 510 min. There were, however, still individuals up to 30 % full after 800 min of starvation in August, and up to 15 % full after 870 min in September.

Table 5.7 shows that, in both August and September 2000, the range of gut fullness values of field-sampled *P. xiphias* males and females was similar at any given depth stratum and time of day. This suggested that they had similar diel feeding patterns. Both males and females were therefore combined in order to increase sample numbers for more robust statistical analysis. Figure 5.20 shows the gut-fullness distributions of individuals collected from the field at different times of the day and night in August and September 2000. In both months, there were significant differences between the median gut fullness values at each time-point (August, Kruskal-Wallis: $H_4 = 12.316$, $P = 0.006$; September, Kruskal-Wallis: $H_4 = 66.043$, $P < 0.001$), suggesting that levels of feeding varied over the diel cycle. In nighttime samples from the top 200 m in both months, the large proportion of individuals that were >50 % full indicated that feeding was taking

		Males		Females	
Time (h)	Depth (m)	GF (%)	*n* (ind.)	GF (%)	*n* (ind.)
BATS143					
01:23	0-110	0-70	13	0-80	20
02:37	0-120	10-40	10	5-60	26
06:23	0-600	20-90	14	30-90	20
06:51	0-800	5-60	23	0-80	29
21:27	0-102	-	-	0-90	5
BATS144					
01:35	0-125	40-80	2	10-100	19
05:20	0-125	90	1	60-100	16
05:52	0-200	-	-	90-100	2
15:33	0-700	0-25	27	0-60	25
20:46	0-237	0-80	34	0-100	70

Table 5.7 The gut fullness range (%) and number of individuals measured (*n*) for *Pleuromamma xiphias* adult males and females collected at different times and depths at BATS in August 2000 (BATS143) and September 2000 (BATS144).

place in the surface layer at this time. Furthermore, the greater proportion of individuals >70 % full in the dawn samples from both months suggested that feeding had continued throughout the night. Secondary maxima appeared to occur around midnight, while in August an increased proportion of emptier individuals in the 02:30 h net-catch implied a post-midnight relaxation in feeding. The 'dawn' sample in September, taken from the top 125-200 m almost 90 min before sunrise, contained a greater proportion of fuller individuals than the 'dawn' sample in August, which was taken from the top 500 m at sunrise. The September daytime sample, collected around 15:30 h from the top 700 m, was found to contain individuals between 0 and 60 % full, with a greater proportion of these individuals being <30 % full. The dusk samples in both August (21:30 h, 0-100 m) and September (20:45 h, 0-230 m), contained a greater number of fuller individuals (up to 100 % full). Most of these individuals were >80 % full.

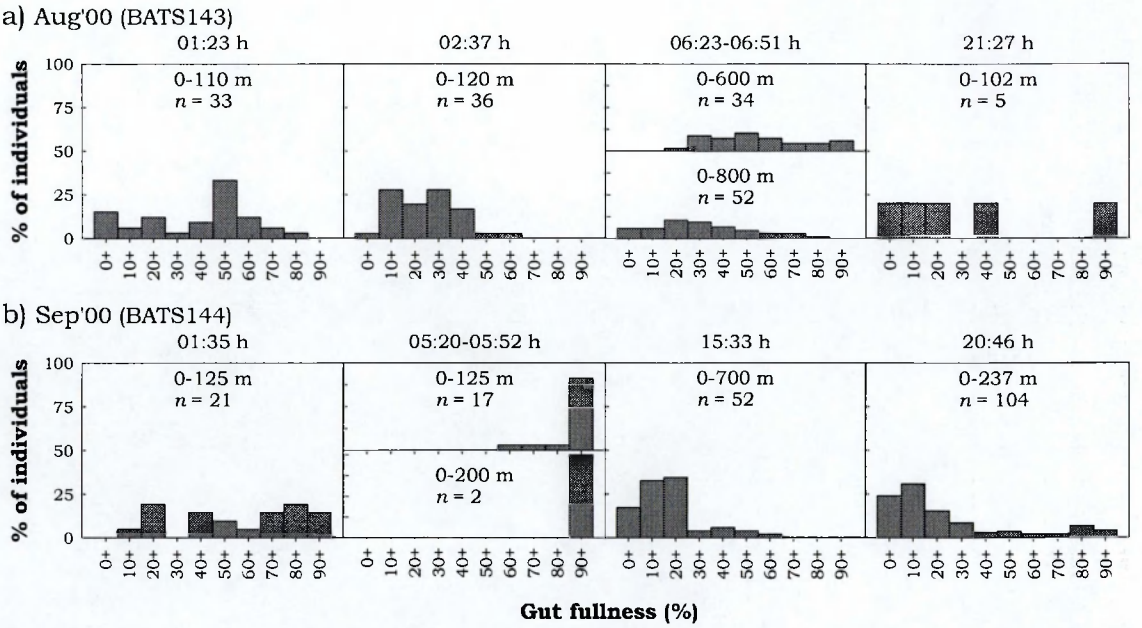

Figure 5.20 The gut-fullness distribution of *Pleuromamma xiphias* (adult males and females combined) collected at different times of the day and night at BATS in a) August 2000 (BATS143), and b) September 2000 (BATS144).

Diel changes in carbon and nitrogen weight

Temporal changes in the carbon and nitrogen weight of starved *P. xiphias* adult males and females were investigated via shipboard time-course experiments conducted in August 2000 (BATS143) and September 2000 (BVAL29) (incubation in 0.45 μm-filtered local surface seawater at 24 and 22.5 °C, respectively). Figure 5.21 shows that there was no systematic pattern of change in either the carbon or the nitrogen weight of size-normalised (3.5 mm-long) individuals (males and females combined) over time in either experiment. ANOVA showed that there were actually significant differences between the time-point means in August (carbon, ANOVA: $F_{5,45}$= 7.36, P <0.001; nitrogen, ANOVA: $F_{5,45}$= 8.217, P <0.001), while no differences were found in September (carbon, ANOVA: $F_{5,95}$= 1.892, P = 0.103; nitrogen, ANOVA: $F_{5,95}$= 1.896, P = 0.102). Variability (*V*) in both carbon and nitrogen weight was high at most of the

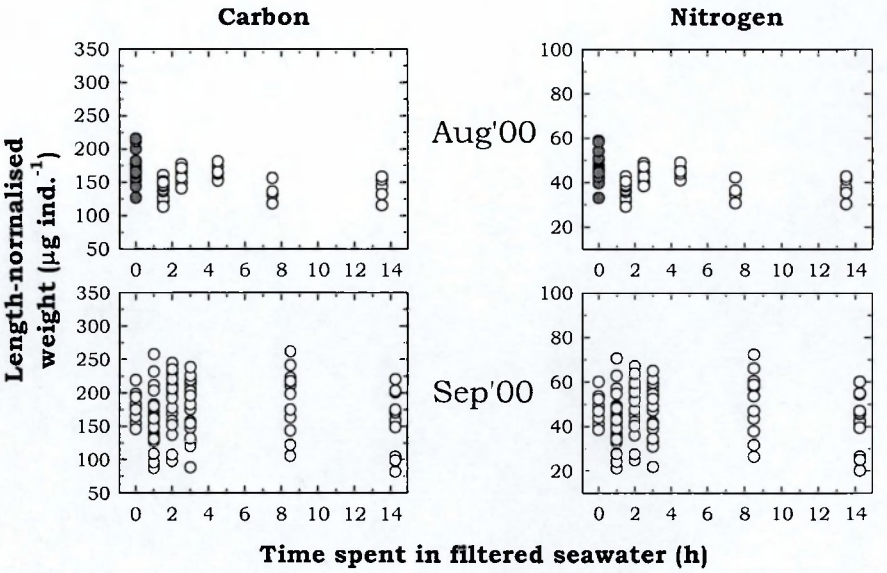

Figure 5.21 The carbon and nitrogen weight of size-normalised (3.5 mm-long) *Pleuromamma xiphias* (adult males and females combined) collected at dawn at BATS in a) August 2000 (BATS143), and b) September 2000 (BVAL29) and maintained for varying lengths of time in the absence of food (incubation in 0.45 µm-filtered local surface seawater at 24 and 22.5 °C, respectively). Filled symbols represent those values measured directly. Empty symbols represent those values derived from dry weight (see Figure 5.16 for the regression parameters used). Values represent the mean of n = 1-5 individuals in August, and n = 1 individual in September.

time-points, ranging from 6 to 15 % in August, and 12 to 30 % in September. All carbon and nitrogen weights, with the exception of time zero in August, were derived from dry-weight measurements as opposed to being directly measured. In August, these measurements were obtained from samples containing 1-5 individuals, while single individuals were weighed in September.

Temporal changes in the carbon and nitrogen weight of starved krill were also investigated during the shipboard time-course experiment conducted in September 2000 (BVAL29). Figure 5.22 shows that there was no systematic pattern of change in either the carbon or the nitrogen weight of size-normalised (10 mm-long) individuals over time in this experiment. ANOVA confirmed that there were no significant differences between the time-point means (carbon, ANOVA: $F_{4,21}$= 1.683, P = 0.191; nitrogen,

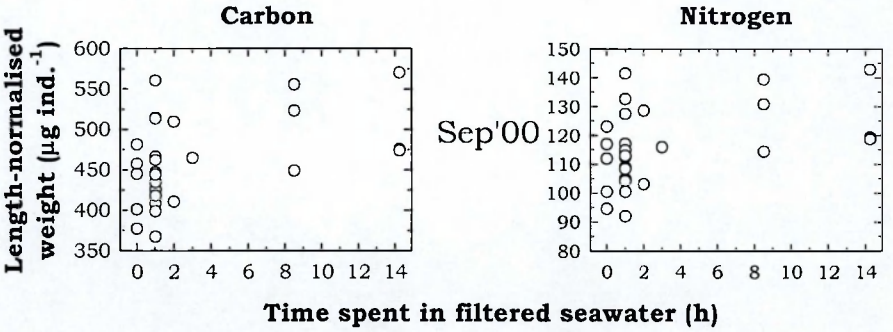

Figure 5.22 The carbon and nitrogen weight of size-normalised (10 mm-long) krill collected at dawn at BATS in September 2000 (BVAL29) and maintained for varying lengths of time in the absence of food (incubation in 0.45 µm-filtered local surface seawater at 24 and 22.5 °C, respectively). Filled symbols represent those values measured directly. Empty symbols represent those values derived from dry weight (see Figure 5.16 for the regression parameters used). Values represent n = 1 individual in all cases.

ANOVA: $F_{4,21}$= 1.511, P = 0.235). Variability (V) in both carbon and nitrogen weight was high at each of the time-points, ranging from 9.8 to 15.6 %. All carbon and nitrogen weights were derived from dry-weight measurements, as opposed to being measured directly.

Figure 5.23 shows the carbon and nitrogen weights of size-normalised (3.5 mm-long) individual *P. xiphias* (males and females combined) collected from the field at various times of the day and night in August 2000 (BATS143) and September 2000 (BATS144). No obvious diel patterns were apparent. Since unequal variances (Levene's: P <0.05) were found between the time-points in both August (nitrogen data only) and September (both carbon and nitrogen data), the median values at each time-point were compared using Kruskal-Wallis in these instances. No significant differences were found between time-points in either August (carbon, ANOVA: $F_{4,68}$= 1.141, P = 0.345; nitrogen, Kruskal-Wallis: H_4 = 6.672, P = 0.154) or September (carbon, Kruskal-Wallis: H_3 = 6.23, P = 0.101; nitrogen, Kruskal-Wallis: H_3 = 7.023, P = 0.071). These findings are not consistent with the idea that individuals were feeding more at night than

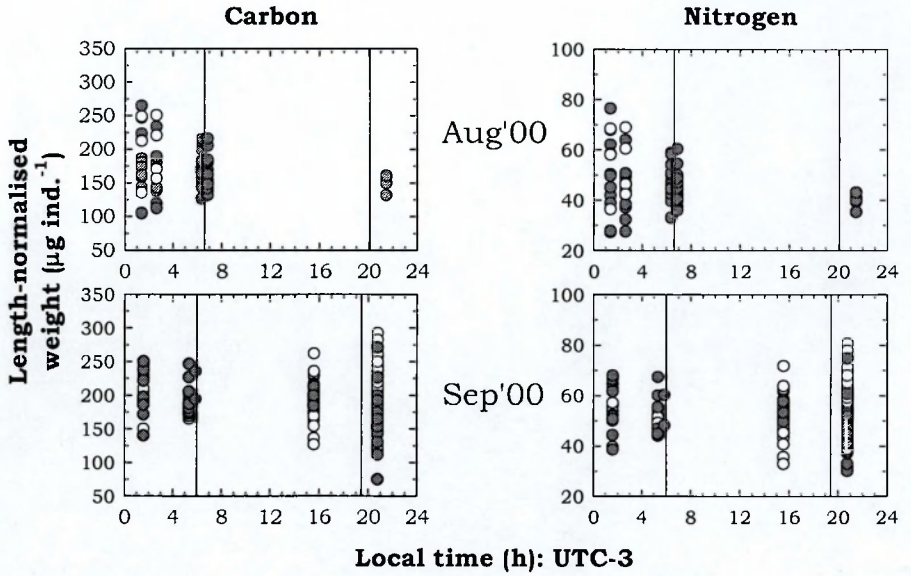

Figure 5.23 Diel differences in the carbon and nitrogen weight of size-normalised (3.5 mm-long) *Pleuromamma xiphias* (adult males and females combined) collected at BATS in a) August 2000 (BATS143), and b) September 2000 (BATS144). Solid lines represent the times of sunrise and sunset. Filled symbols represent those values measured directly. Empty symbols represent those values derived from dry weight (see Figure 5.16 for the regression parameters used). Values represent the mean of n = 1-5 individuals in August, and n = 1 individual in September.

during the day. Variability (V) in both carbon and nitrogen was high at each time-point, ranging from 7 to 30 % in August and 10 to 20 % in September. In August, greater variability was found at those time-points that consisted, in part, of carbon and nitrogen weights that had been derived from dry weight.

Figure 5.24 shows the carbon and nitrogen weights of size-normalised (10 mm-long) individual krill collected from the field at various times of the day and night in August 2000 (BATS143) and September 2000 (BATS144). A marked diel pattern was evident in August. There were significant differences between both the carbon- and nitrogen-weight means at each time-point (carbon, ANOVA: $F_{2,71}$= 16.095, P <0.001; nitrogen, ANOVA: $F_{2,71}$= 15.662, P <0.001), with both carbon and nitrogen weight being higher at dawn than at either dusk or during the night (Tukey: P <0.05) (dawn-dusk difference:

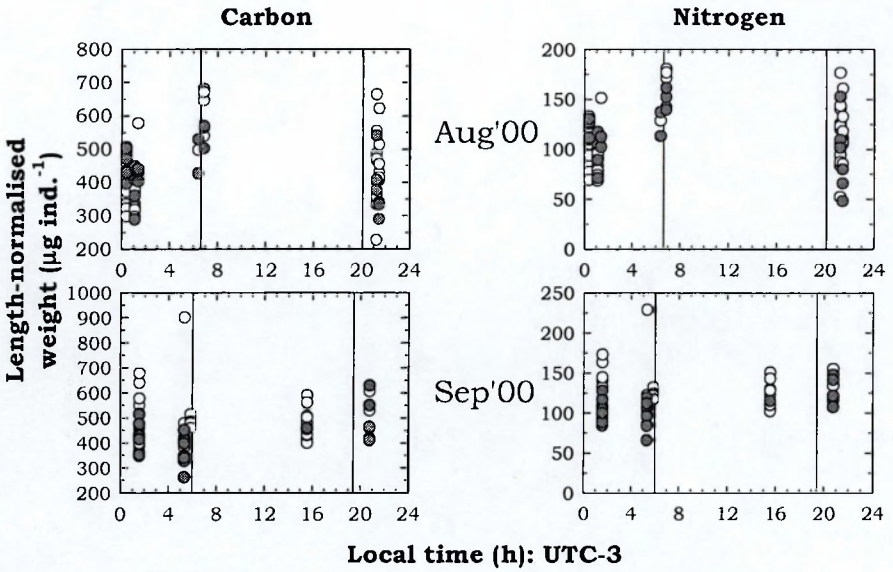

Figure 5.24 Diel differences in the carbon weight and nitrogen weight of size-normalised (10 mm-long) krill collected at BATS in a) August 2000 (BATS143), and b) September 2000 (BATS144). Solid lines represent the times of sunrise and sunset. Filled symbols represent those values measured directly. Empty symbols represent those values derived from dry weight (see Figure 5.16 for the regression parameters used). Values represent n = 1 individual in both months.

carbon = 23.5 %, nitrogen = 27.0 %). This pattern is consistent with the idea that individuals were feeding more at night than during the day. Variability (V) was high at each time-point, ranging from 14.6 to 24.0 %. No diel pattern was evident in September. Since unequal variances (Levene's: P <0.05) were found between time-points for both carbon and nitrogen weight, the median values at each time-point were compared using Kruskal-Wallis. No significant differences were found (carbon, Kruskal-Wallis: H_3 = 5.526, P = 0.137; nitrogen, Kruskal-Wallis: H_3 = 7.618, P = 0.055), a pattern that is not consistent with the idea that individuals were feeding more at night. Variability (V) in both carbon and nitrogen was high at each time-point, ranging from 12.9 to 31.9 %.

5.3.5 Statistical assessment of the success of ZOOFLUX in the Sargasso Sea

Table 5.8 shows the statistical success of the second field-application of ZOOFLUX,

Date	Element	n	δ %	V %	ANOVA: P	β %	n_{min}	δ_{min} %
P. xiphias								
BATS143	Carbon	21	10	11	0.109	77	36	14
	Nitrogen	21	13	11	0.053	62	24	14
BATS144	Carbon	62	<1	16	0.931	95	45000	11
	Nitrogen	62	-2	17	0.694	95	2150	12
Krill								
BATS143	Carbon	18	23	19	0.001	n/a	12	20
	Nitrogen	18	27	22	<0.001	n/a	12	22
BATS144	Carbon	12	-14	24	0.251	91	88	39
	Nitrogen	12	-16	22	0.201	88	70	38

Table 5.8 The statistical 'success' of ZOOFLUX at BATS (see section 2.3.8), based on the carbon and nitrogen weight of length-normalised migrants (3.5 mm-long *Pleuromamma xiphias*, adult males and females combined; 10 mm-long krill) collected at dawn and dusk. n = the mean of the number of samples collected at dawn and at dusk. δ = the dawn-dusk difference in carbon or nitrogen weight. V = the mean coefficient of variation of the carbon or nitrogen data from both dawn and dusk. β = the probability of committing a Type II error (when ANOVA: P >0.05). n_{min} = the minimum number of samples required in order to detect a significant δ (ANOVA: P $\leq$0.05). δ_{min} = the minimum detectable dawn/dusk difference.

based on *P. xiphias* and krill collected at BATS in August and September 2000 (see section 2.3.8 for an explanation of the calculations used).

The number of samples available for the dawn-dusk comparisons (n, expressed as the mean of the number collected at both dawn and dusk) were low in most cases (12 to 62 samples), while the variability in the carbon and nitrogen measurements (V, expressed as the mean of the coefficients of variation at both dawn and dusk) was relatively high (11 to 24 %). In three out of eight comparisons, the dawn-dusk difference (δ, expressed as a percentage change) was actually seen to be negative (i.e. carbon and/or nitrogen weight actually seemed to have increased during the day). In six out of eight comparisons, δ was found to be non-significant (ANOVA: P >0.05), and the probability that a Type II error had been made (β) was high in each case (62 to 95 %). The

variability (V) in the data was relatively high in each of these instances (11 to 24 %), and sample numbers often low (12 to 62 samples).

Overall, the minimum number of samples that would have been required (n_{min}, expressed as the mean) in order to conclude that the observed dawn-dusk differences were significant (ANOVA: $P \leq 0.05$) ranged widely (12 to 45000 samples). In only two instances was this number less than the number of samples actually collected, and in half the cases the number required was not realistically obtainable (>50 samples). The magnitude of n_{min} related less to either the sample size (n) or the variability in the data (when expressed as V), and more to the particular source of the variability. Specifically, the higher the error MS in relation to the to the groups MS (see section 2.3.8), the higher the value of n_{min}, and *vice versa* (recall that Equation 2.11 employs only error MS as the variability term). That is to say, the variability within the data at either dawn or dusk was more important in deciding the minimum number of samples required than the variability between the data at each of these time-points. Finally, the minimum dawn-dusk difference that would have been detectable (δ_{min}) in each case ranged from 11 to 39 %.

5.4 Discussion

As with the first field study in the Clyde Sea (Chapter 4), fieldwork carried out in the Sargasso Sea was primarily aimed at gaining a better understanding of the active flux of carbon and nitrogen in the marine environment. While less accessible than Inchmarnock Water, the BATS site is more representative of the typical oceanic situation, and therefore arguably more relevant as a site for such a study. Previous studies have revealed the composition of the interzonal migrant community at BATS (see section 5.1.5 for references), and what appear to be the two most significant migratory taxa

from these investigations were collected during the present study (i.e. *Pleuromamma xiphias* and krill). Data from the standard BATS-programme net tows (Figure 5.11) revealed that interzonal migration was a feature of the ecosystem during the present sampling campaign, and provided information on the size-composition and dry-weight biomass of the migrant community. These data were used in conjunction with the depth-integrated WP-2 net tows made during the present study (Figure 5.7) to estimate the numbers of *P. xiphias* and krill migrating into and out of the top 200 m each day (see Table 5.3 and relevant text), and the timing and amplitude of these movements (see Figure 5.12 and relevant text). Despite the rich history of oceanographic research in this region (see section 5.11), this higher-resolution, yet simple approach to DVM at BATS does not appear to have been taken to date. Moreover, the demonstration of a biological application to the acoustic data that has been almost continuously gathered at the BTM mooring site since 1994 (Figure 5.13) is intended to alert other interested users to the existence of a valuable source of data.

As with *Calanus* in Inchmarnock Water (see section 4.3.3), biometric measurements of *P. xiphias* and krill at BATS (Figures 5.14 to 5.17) revealed interesting patterns of individual physiological variability that were often difficult to interpret or reconcile with other findings. Again, this information, along with other environmental measurements (Figures 5.8 to 5.10), now allows a number of active-flux related questions to be posed: (1) Was the physical environment conducive to an active flux?; (2) How and why were migrants behaving as they did, and what were the consequences of this to the ecosystem?; (3) Was an active export flux of carbon or nitrogen actually occurring at this site, and, if so, could it be quantified accurately using the present dataset? To these three questions can also be added a fourth: (4) Are we now better able to address some of the issues that have arisen from previous studies in the region? These

questions are addressed in turn below.

5.4.1 The physical environment

Bathymetry

The enormous depth of the water column (~4680 m), and the presence of two potential barriers to upward fluxes in the form of the seasonal and permanent thermoclines (from ~10 to ~160 m, and from ~400 to ~1200 m, respectively), means that any material that does find its way to depth is likely to remain there for a significant period of time. Indeed, studies in other areas of the world's oceans have shown that deep-water POC is relatively young, indicating that this is the dominant form in which material is exported, while deep-water DOC is much older (~6000 y old on average), indicating that this is the main form in which exported material is stored at depth. The BATS site is therefore representative of the potentially huge importance of the oceans in mitigating the climatological effects caused by anthropogenic emissions of greenhouse gases such as CO_2, CH_4 and nitrogen oxides. It is for this very reason that a biogeochemical time-series study (i.e. BATS) was established here.

Hydrography

The seasonal changes in the structure of the water column at BATS have been well studied (see section 5.1.2 for description and references). It has therefore been well established that the combination of high temperatures and low wind-stress during the summer months (April to October) results in strong stratification. During this period, the presence of a seasonal pycnocline (from ~10 to ~160 m) means that any material carried beneath this boundary is less likely to be able to return to the surface layers, and from there potentially back into the atmosphere. The BATS site therefore possesses the

classic physical attributes necessary for significant export flux to occur. During the present study, which was carried out in late summer, the physical structure of the water column was certainly found to reflect this flux-conducive regime (Figure 5.8).

As in the Clyde Sea, however, this stratification breaks down in the winter months (the shallower seasonal pycnocline, but not the deeper permanent pycnocline), and previously sequestered material has the opportunity to return to the surface. As Longhurst *et al.* (1990) discussed, while an active transport may still occur in the winter months, it will not have the same significance if a physical barrier to mixing is no longer present. The form (dissolved or particulate) and vertical distribution of sequestered material, and the depth and intensity of deep-mixing events, are important in deciding how much of this material is returned to the surface layer each year. In terms of striking a balance between the 'health' of the pelagic ecosystem for the following year, and the climatological effects of excess greenhouse gases, it is important that enough nutrients are returned to 'kick-start' the annual cycle of primary production (i.e. new production), while not so much are returned that the ocean 'exhales' previously 'inhaled' gases. To understand and predict the dynamics of this balance, we need to understand the processes controlling both the vertical distribution of biogeochemically-important material and the depth and intensity of deep mixing events. A potential feedback mechanism is apparent here: the levels of greenhouse gases influence the climate, which influences the dynamics of the upper ocean, which influences the levels of greenhouse gases.

5.4.2 Mesozooplankton behaviour and ecosystem consequences

The relevance of understanding the behaviour of the mesozooplankton community in the context of an active flux study was explained in section 4.4.2. In the present study at

BATS, the behaviour of the mesozooplankton community (>200 μm) was investigated through net tows and acoustics (at 150 kHz). Historically, net-catch data have shown, as is typical of tropical and subtropical open-ocean environments, that the zooplankton community at BATS is highly diverse and both temporally and vertically variable in terms of species composition and numerical abundance (e.g. Sutcliffe, 1960; Deevey & Brooks, 1977; Madin *et al.*, 1996). It is also known from previous studies (e.g. Steinberg *et al.*, 2000; Madin *et al.*, 2001) that a number of species, primarily copepods and krill, undertake interzonal DVM in this region (see section 5.1.5). While no studies to date appear to have identified the exact timing, amplitude and causes of these migrations at BATS (but see Buskey *et al.*, 1989, for sites in the northern and southern Sargasso Sea), the fact that migrants are almost never found in the surface waters by day (D.K. Steinberg, pers. comm.) strongly suggests that they are typically performing a 'normal' DVM pattern (NDVM) of up at dusk and down at dawn. Figure 5.13 showed that acoustic techniques have the potential to enhance our understanding of DVM at BATS. To date, very little attention has been directed to the extraction of biological patterns from the BTM ADCP data. Given the recognised usefulness of ADCPs in zooplankton studies (see section 3.4.2), and the lack of time-resolved information on zooplankton DVM at BATS, it is apparent that a potentially important dataset awaits analysis.

Interpreting the net-catch and acoustic data

The various issues surrounding the interpretation of net-catch data were discussed in section 4.4.2. While it is true that the low spatial and temporal resolution of the BATS time-series tows (i.e. replicate tows by day and by night in the top 200 m) does not allow the exact timing and amplitude of vertical movements to be resolved, the

likelihood that interzonal DVM at this site follows a relatively simple pattern of up at dusk and down at dawn (D.K. Steinberg, pers. comm.) means that day/night differences in the biomass calculated from these tows are likely to be a fairly accurate representation of the biomass of the vertically migrating community (although one must still consider the issues of patchiness and horizontal advection). Similarly, while the integrated tows used to collect specimens for the present study did not allow the exact depth at which individuals had been residing to be known, a fair degree of behavioural information was extracted by process of elimination and the application of literature estimates of swimming speeds (see Table 5.3 and Figure 5.12, and associated sections in text).

As also discussed in section 4.4.2, while the ADCP is capable of providing biological information on more highly-resolved time and space scales to net tows, interpreting the data from such instruments is subject to a number of often-complex considerations. A 150 kHz ADCP has been deployed at 200 m depth on the BTM mooring since 1994. Assuming an average speed of sound through water of 1500 m s^{-1} (Equation 3.4 is no longer valid with the higher water temperatures at BATS), the wavelength of a sound pulse at 150 kHz will be 10 mm (Equation 3.3). This means that the majority of the backscatter evident in Figure 5.13 is most likely to have been caused by organisms ≥10 mm. Of the interzonal zooplankton listed by Steinberg *et al.* (2000) and Madin *et al.* (2001), all except the copepods should consist of at least some individuals which are large enough to be acoustically detectable at this frequency. However, as shown by Equation 4.2, it is entirely possible that higher concentrations of smaller species such as *P. xiphias* will also be detectable at 150 kHz.

Mesozooplankton DVM behaviour and the potential for an active flux to occur

It is likely that *P. xiphias* and krill would each have been contributing to an active flux during the present study at BATS as a consequence of their behaviour. The following discussion relates to both of these migrants.

The vertical migration strategy of P. xiphias and krill

The net-catch data summarised in Figure 5.12, in conjunction with the CTD data (Figure 5.8), clearly showed that both *P. xiphias* and krill were undertaking interzonal DVM at BATS in August and September 2000. The nighttime increases seen in the biomass of the 2-5 mm size fraction in the top 200 m (Figure 5.11) indicated that *P. xiphias* adults were probably a significant part of the migrant community in both months. However, the lack of a DVM signal in the >5 mm size fraction in August, and the presence of only a relatively small signal in September, indicates that krill, at least those >5 mm, were not such an important component of the migrating community at this time of year. While these data are not as well resolved as those for *Calanus* in the Clyde Sea (see section 4.3.3), previous net tows at this site have shown that almost no migrants remain in the upper 200 m during the daylight hours (D.K. Steinberg, pers. comm.). We can, therefore, be fairly sure that *P. xiphias* and krill were swimming up and down through the pycnocline around dusk and dawn, respectively. This is in agreement with the timing and amplitude of DVM estimated from the data presented in Figure 5.12 and described in section 5.3.4. That is to say, both *P. xiphias* and krill were likely to have been performing a simple and classic pattern of NDVM at BATS during the present study.

Causes of the vertical migration of P. xiphias and krill

Are there any indications as to why *P. xiphias* and krill behaved as they did at BATS? General factors governing the behaviour of animals were discussed in section 4.4.2. As with *Calanus*, *P. xiphias* and krill at BATS probably ascended into the surface layer to feed on the rich supply of phytoplankton there (see section 5.1.3). The presence of these phytoplankton in the top 200 m during the present study is evidenced by the profiles of chlorophyll *a* fluorescence (P. Lethaby, pers. comm.) and primary production (Figure 5.9). Both *P. xiphias* and krill are known to feed on a variety of phytoplankton at BATS, in particular diatoms (despite their generally low abundance: see Schnetzer & Steinberg, 2002b), and one might expect the nighttime vertical distributions of these interzonal migrants to mirror those of their preferred food items. When present at BATS, diatoms are found from the surface to around 200 m at the most, with concentration maxima typically between 50 and 150 m (see Figure 14a in Steinberg *et al*, 2001). However, this 8 y time-series also showed that diatoms are often rare or absent in August and September. It is therefore possible that both *P. xiphias* and krill were also feeding carnivorously at this time. Indeed, Schnetzer & Steinberg (2002b) showed that the diet of *P. xiphias* was more notably carnivorous in autumn and early winter, with prey items for both *P. xiphias* and krill including primarily protozoans and crustaceans, but also other groups such as chaetognaths and cnidarians. The observation that *P. xiphias* did not ascend shallower than ~75 m (from Figure 5.12) suggests that this species was associating with the deep chlorophyll maximum layer (DCML) at night, which was around 100 m in both August and September 2000 (P. Lethaby, pers. comm.). This is certainly in agreement with Roman *et al.* (1993), who found that the migrant community at BATS concentrates just above the DCML. This association might be either direct (i.e. to feed on the phytoplankton material present) or indirect (i.e. to feed

on smaller metazoan prey which might themselves be directly associating with the phytoplankton). The observation that krill ascended shallower than this (from Figure 5.12) may be a product of resource partitioning between themselves and *P. xiphias*, and also suggests that *P. xiphias* was not prey for larger krill. As also observed with *Calanus* in Inchmarnock Water (section 4.4.2), some *P. xiphias* individuals were found to have food material in their stomachs while at depth during the day (Figure 5.20). Unlike *Calanus*, it is unlikely that this food had been ingested in the surface layer (but see the discussion of protracted gut passage time, below), and much more likely that detritivory and/or carnivory was occurring at depth during the day. Schnetzer & Steinberg (2002b) showed that *P. xiphias* (and also krill) ingest detritus at BATS, commonly marine snow from discarded larvacean filter houses. The fact that individuals needed to feed during the daytime suggests that the full dietary requirements were not being met through nighttime feeding alone. The fact that *P. xiphias* (and indeed also krill) undertook the substantial upward journey into the surface layer every day indicates that any food at depth was not sufficiently nutritious to maintain a healthy state for any length of time.

Descent at dawn was probably to enable the avoidance of visual predators in the sunlit surface layer. Avoidance of harmful UV rays is unlikely, since daytime depth distributions are deep, whilst UV is absorbed within the first few m of the water column (e.g. Leech & Williamson, 2001). Similarly, if descent was to partition their food resources in either space or time, for example, one might also expect either a shallower daytime distribution or no migration at all. The fact that individuals were moving to depths of 400 m or more, and expending significant amounts of energy, strongly suggests that this was an adaptive escape mechanism. As discussed above, the daytime depth-distribution of vertical migrants will be important in deciding the depths to which

surface-derived material is actively exported, and hence the likelihood of it being returned during subsequent winter mixing. Buskey *et al.* (1989) showed that the daytime depths of *P. xiphias* in both the more turbid northern Sargasso Sea, and the less turbid southern Sargasso Sea, correlated significantly with light intensity. However, it was also found that light intensities varied quite considerably over the daytime depth range, and that the mean depth (459 to 634 m) of the population was always found to be 300 to 400 m shallower than the depth predicted from the experimentally-derived photosensitivity threshold of this species. It was therefore suggested that there was either marked individual variability in photosensitivity, or that factors other than light were also involved in controlling daytime depths. The potential factors discussed included hydrostatic pressure, temperature, salinity, the rapid descent of isolumes causing photoadaptation and a reduction in photosensitivity, the size of individuals and their descent speeds. Regarding temperature, it is possible that the ~18 °C STMW layer, which extended from 160 to 400 m during the present study (Figure 5.8), might be influential in deciding the daytime depth of migrants. It is certainly interesting to note that the base of this layer corresponds with the shallowest depth of daytime migrants, which might suggest that, for metabolic reasons, temperatures <18 °C need to be maintained during daytime periods of reduced feeding (recall the metabolic causes of DVM suggested by McLaren, 1963, 1974; see section 1.1.6). Regarding body size, previous observations that juvenile *P. xiphias* were found shallower than adults during the day, and that a smaller species, *P. gracilis*, was found shallower still (see Figure 5 in Buskey *et al.*, 1989), lend weight to the validity of the predator evasion hypothesis (e.g. Zaret & Suffern, 1976), i.e. that larger individuals, which might be more visible to predators, need to swim to deeper, darker depths before they are no longer detectable (e.g. Angel, 1985; Hays *et al.*, 1994, de Robertis, 2002).

5.4.3 Was an active flux occurring, and could it be measured with the current dataset?

Through investigating the active flux at BATS, this study, by necessity, has required an understanding to be gained of a variety of physical and biological parameters at this site. Although the fieldwork and processing methods were simplistic, a certain amount of new knowledge has been gained, and subsequently utilised to build upon the findings from previous studies. In particular, calculations have been provided here which shed new light on the timing, amplitude and biomass of migrating *P. xiphias* and krill at BATS, and a discussion provided of the potential causes of these movements. Furthermore, this study has also highlighted a number of gaps that exist in our knowledge of DVM behaviour at this site, despite the extensive history of research in the region. For example, it is apparent that we still know very little about DVM at BATS on a species-specific level: little is known about the seasonal changes in the migrating biomass of each species, nor the timing, amplitude and causes of these movements. A species-specific, seasonal study of DVM at a globally important site such as BATS would therefore be timely, not only to carry research of this ubiquitous phenomenon into a new era (see Hays, in press, for examples of some of the directions we might take), but also to investigate in more detail the role of zooplankton DVM in the sequestration of biogeochemically important elements in the ocean's interior. Based on the information gathered thus far, we can now address the question of whether an active flux was occurring at BATS, and whether this potential flux could have been successfully measured with the present dataset.

Evidence for flux-conducive behaviour

Given that *P. xiphias* and krill were probably performing interzonal NDVM, and the

likelihood that they were feeding more in the top 200 m at night than at depth during the day (see discussion above), one can be fairly sure that an active export flux does occur at BATS during the stratified months of the year. Indeed, all of the other active flux studies carried out at this site have agreed that interzonal DVM is likely to be causing an export of material from the surface layer (Dam *et al.*, 1995; Steinberg *et al.*, 2000, 2002; Schnetzer & Steinberg, 2002a). Unlike *Calanus* in the Clyde Sea, which may have been moving to and from the mixed layer more than once during the diel cycle, the DVM behaviour of these open-ocean species conforms more closely to the classically-described pattern of up at dusk and down at dawn (i.e. NDVM).

The gut fullness data have not been fully discussed thus far in relation to the role of migrants in the export of surface-derived material. Starvation experiments suggested that the gut passage time (GPT) of *P. xiphias* was particularly long (on the order of hours: see Figure 5.19). This supports previous studies that have found the GPT of interzonal migrants to be significantly longer than that of epiplanktonic species (e.g. Morales *et al.*, 1993; Atkinson *et al.*, 1996), and agrees with Schnetzer & Steinberg (2002a) that *P. xiphias* will therefore be able to contribute to the active flux via defaecation at depth. While a significant proportion of the guts was voided within 150-180 min (a 30-35 % reduction since time zero), the fact that some individuals were still up to 50 % full after 250 min, and some up to 15 % full after 870 min, implies a remarkably long GPT. The use of filtered seawater and faecal pellet traps allowed feeding on particulates >0.45 μm to be discounted as the cause of this apparently long GPT, although the possibility that cannibalism had occurred during the August experiment cannot be completely overruled. Schnetzer & Steinberg (2002a) reported a GPT of 99-265 min for *P. xiphias* at BATS, but also found that a mean of 8 % of the initial gut pigment still remained after 12 h of starvation. This suggests that the guts are

not always completely voided, and may explain the continued presence of material in the guts after 14.5 h of starvation in the present study. The much shorter GPT of 25-30 min reported by Arashkevich (1977) for *P. xiphias* highlights the potential problems with obtaining accurate ecological information from laboratory experiments (see e.g. Ikeda, 1977). Continuous feeding was allowed to occur during the latter study, which, given the fact that the rate of faecal pellet production increases linearly with increases in ingestion rate (Mauchline 1998), might help explain why GPT was so much shorter than in the starvation-based experiments carried out here and by Schnetzer & Steinberg (2002a). Different food conditions can also be expected to influence GPT (Mauchline, 1998).

The gut fullness of field-sampled individuals (Figure 5.20) suggested that the feeding pattern of *P. xiphias* might not have simply involved continuous feeding throughout the night followed by starvation during the day. Firstly, the levels of feeding may have oscillated during the night. Variable nighttime feeding rates will influence the number of faecal pellets produced in the surface layer, and therefore, at least when there is sufficient zooplankton biomass, the magnitude of the sinking particle flux. For a 16 h night, as observed in September, and a GPT of 3 h, for example, the maximum number of full gut-loads released (if feeding continuously) will be ~5 gut loads ind.$^{-1}$. As Schnetzer & Steinberg (2002a) showed, defaecation at the surface by feeding migrants can contribute significantly to the passive particle flux (exceeding the active POC flux by ~10-fold in their study), although the indications from the present study that a single gut-load of material in *P. xiphias* contains little material relative to the whole individual (Figure 5.15) show that defaecation is not the major loss term for material in this species. In fact, it seems more likely that larger migrants such as krill and salps, which produce larger, faster-sinking pellets, will be more important in augmenting the passive

flux of POM. Secondly, the presence of individuals up to 60 % full at depth in the middle of the afternoon (~8.5 h after sunrise) suggests that detritivory and/or carnivory was occurring at depth, while the fact that most individuals were <30 % full shows that levels of feeding at this time were lower than at the surface at night. Given the potentially long GPT of this species, there is a possibility that this material originated from food ingested at the surface during the night, but the observations by Schnetzer & Steinberg (2002a) that *P. xiphias* can feed on both detritus and smaller metazoa makes it more likely that feeding was indeed occurring at depth. This will have potential consequences for the magnitude of the active flux, and the ability to quantify it with the present dataset.

GPT could not be estimated in the same way for krill, however, since the gut contents were not visible through the body wall. However, Schnetzer & Steinberg (2002a) found that *T. aequalis/E. brevis* at BATS had a GPT of 15-99 min. This shorter GPT relative to *P. xiphias* would mean that more faecal pellets could be produced in the surface layer at night. This supports the previous suggestion (above) that krill might be more important than copepods in the production of sinking POM, especially given that individual pellets will be larger and will sink faster, meaning that they will lose less material to the water column during descent and stand a better chance of penetrating the pycnocline. A shorter GPT also means that the contribution of krill to the active POM flux might not be as much as the contribution by *P. xiphias*. In either case, Schnetzer & Steinberg (2002a) concluded that the active POM flux at BATS is low, although it will assume greater importance during periods of high migrating biomass. The net-catch data (Figure 5.12) indicated that krill might have been performing midnight sinking behaviour. If this were true, this might enhance the active flux if individuals passed through the pycnocline during this period. It would certainly be interesting to find out

whether midnight sinking does occur at BATS (in any of the migrant species), and whether it will have an impact on the active flux.

Quantifying the active flux according to ZOOFLUX

With such marked NDVM behaviour, one would expect there to have been measurable differences between the carbon and nitrogen weight of migrants collected at dawn and dusk, such that the active fluxes of carbon and nitrogen could be quantified according to ZOOFLUX. It is therefore surprising that a significant dawn-dusk difference in carbon or nitrogen weight (ANOVA: $P \leq 0.05$) was not found in most cases (see section 5.3.5). The statistical assessment (Table 5.8) revealed high variability and low sample numbers as the primary factors. These are the same factors attributed to the difficulties also encountered with applying this technique in the Clyde Sea, despite the very different physical and biological regimes between these sites, suggesting that there is a common obstacle to this *in situ* method of quantifying the active flux. Since the carbon- and nitrogen-weight dataset at BATS was similarly reinforced by dry-weight derived values for statistical purposes, the issues surrounding the use of dry weight as a proxy for carbon and nitrogen weight are again the same as those discussed in section 4.4.3. It is tempting to suggest that relative inexperience in the field and laboratory were partly responsible for the inability to detect any significant diel changes. However, the required sampling and processing protocols were relatively simple to carry out, and the data obtained likely to have been sufficiently accurate (see Table 5.5). Furthermore, an analysis (not shown here) of unpublished data collected in a similar manner by G.C. Hays at BATS in August 1998 (during cruise BATS119) revealed similar issues regarding low sample numbers and high variability. This suggests that there are factors other than human error to consider.

The first source of error concerns the exact times at which the 'dawn' and 'dusk' samples were obtained, and the fact that the actual loss of material at depth was probably not being measured. If one assumes that changes in elemental composition may well be rapid when switching from active nighttime feeding to less active, or non-existent, daytime feeding and *vice versa*, it becomes apparent that the collection of individuals as close as possible to the times at which they cross the pycnocline is imperative when attempting to quantify the active flux according to ZOOFLUX. The first problem with the present sampling campaign was that an opening/closing net was not used, so that it is not known if migrants were above or below the pycnocline when collected, and therefore whether they were collected at the correct times. This is therefore a logistical issue which, in this case, could not be avoided due the lack of a suitable opening/closing net. The second problem concerns the depth of the pycnocline and the associated duration of net tows. At BATS, where the base of the pycnocline is at ~160 m, net tows will take at least 40 min. Assuming individuals were caught at the deepest extent of the tow, then they would have been present in the cod-end for 20 min before reaching the boat, followed by at least 10 min of processing before preservation (i.e. freezing). It is therefore likely that, no matter how close to the optimum time the samples are collected, significant amounts of carbon and nitrogen may well be lost before the samples are measured. We can also use gut fullness (for *P. xiphias* only) and the proximity of the net tows to sunrise and sunset to indicate how close the samples from the present study were to the optimum dawn/dusk time-points. The high proportion of emptier individuals at 'dawn' in August, and the fact that the sample was collected from 0 to 500 m at sunrise, suggested that this sample had been collected a little late. The September 'dawn' sample, collected ~90 min before sunrise from the top 125/200 m, and containing a greater proportion of fuller individuals, was probably much

closer to the dawn optimum. In both August and September, the 'dusk' samples were collected ~1.5 h after sunset and contained a large proportion of fuller individuals, suggesting that they were taken a little late in both cases.

The second source of error, for *P. xiphias* at least, relates to the varied body condition of individuals during each cruise, as indicated by the length/weight relationships illustrated in Figure 5.14. For individuals with the same prosome length, the body weight (dry, carbon or nitrogen) was somewhat variable, indicating that the samples collected during any one cruise consisted of individuals with variable feeding histories. This highlights a classic problem of sampling in open-water environments, namely the fact that one cannot always sample from the same population over time. However, even if we were able to sample from the same population, it is entirely possible that individual variability in body condition would have still been high enough to present statistical problems. Interestingly, the C:N of *P. xiphias*, another measure of body condition, was seen to be uniform (Figure 5.17), even when considering both sampling cruises together. The fact that the lipid/protein relationship was similar in all individuals, while the body weight at a given length was highly variable, suggests that some other constituent of *P. xiphias* was varying in line with differential feeding success. The non-normal length distribution noted during each cruise lends weight to the idea that the combined samples consisted of individuals from separate populations. The body condition of krill appeared to be much more uniform, such that the main inaccuracy in quantifying the daytime losses of carbon and nitrogen probably lay in the timing and duration of the net tows.

The third source of error is that it was not always possible to obtain individuals in any great numbers. This might seem somewhat strange to someone who has never towed a plankton net, and who might expect that tows would always come up teeming

with animals. However, the reality is often different, especially when animals must be collected at one specific time so that nets cannot simply be fished repeatedly until enough samples are obtained. For a statistically-based protocol such as ZOOFLUX, the high levels of variability that one comes to expect from animals in a dynamic habitat requires that large numbers of samples be collected to overcome this issue. Net hauls devoid of target organisms are therefore a major problem when using ZOOFLUX, and one that cannot easily be remedied, at least if continuing to employ conventional net-sampling methods.

In conclusion, while the classic and pronounced NDVM behaviour of both *P. xiphias* and krill at this site indicates that they should be causing an active export flux of biogeochemically important elements, the ability to measure this flux using ZOOFLUX is hampered by a number of logistical and biological factors which may, or may not, be readily mitigated.

5.4.4 Consequences for carbon imbalance at BATS

As Steinberg *et al.* (2001) discussed, three pressing questions have arisen as a result of studies at BATS. These represent uncertainties in the nature of the oceanic carbon cycle, and need to be resolved if we are to understand the role of the oceans in the global cycling of carbon. The first is a disagreement between the magnitudes of the *f*-ratio (the fraction of primary production fuelled by nitrate from below the mixed layer as opposed to nitrate and ammonium recycled in the mixed layer) as estimated by either short-term or long-term measurements (Jenkins & Goldman, 1985). The second is the observation that the ratios of carbon, nitrogen and phosphorus do not appear to be at traditional "Redfield" levels (Redfield *et al.*, 1963), which leads one to ask what mechanisms are causing this deviation from the norm (see Michaels & Knap, 1996, for a discussion of

this topic). The third is an apparent carbon imbalance (Michaels *et al.*, 1994): the spring-autumn reduction in the total amount of carbon in the top 150 m has been found to be three times greater than the total amount of carbon being exported from this depth interval via the sinking of large particles, the air-sea exchange of CO_2, vertical mixing, downwelling, horizontal advection and zooplankton DVM.

It is evident that data from the present study may be able to shed some light on this last question, namely that of the 'missing' carbon. Michaels *et al.* (1994) suggested that either sediment traps are missing 80 % of the sinking particles, or that 70 % of this carbon is being removed by horizontal advection (or a mixture of the two processes). It was concluded that sediment trap inaccuracies were the prime cause of this carbon imbalance. More recently, it has been suggested that other unresolved processes may also be of importance, including eddy pumping (McGillicuddy & Robinson, 1997), zooplankton DVM (Steinberg *et al.*, 2000, 2002), and the passage of hurricanes (Bates *et al.*, 1998). According to Michaels *et al.* (1994), we need to account for an additional export of 96 mg C m^{-2} d^{-1} in order to close the carbon imbalance at BATS. In their calculations, they used an active flux estimate of 6 mg C m^{-2} d^{-1}, based on the respiratory carbon-flux values estimated in this region by Longhurst *et al.* (1990). Given that Dam *et al.* (1995) found a maximum respiratory carbon-flux at BATS of 41 mg C m^{-2} d^{-1}, it seems entirely possible that zooplankton DVM can account for a significant proportion of the imbalance. Recognising this, Steinberg *et al.* (2000) added their assessments of the excretory carbon-flux (DOC excretion = 31 % respiration) to the mean respiratory carbon-flux measured by Dam *et al.* (1995) (= 14.5 mg C m^{-2} d^{-1}), thereby closing the imbalance from 96 to 83 mg C m^{-2} d^{-1}. If we continue this exercise by adding the mean defaecatory POC-flux measured by Schnetzer & Steinberg (2002a) (= 0.94 mg C m^{-2} d^{-1}), the imbalance can now be closed to 82 mg C m^{-2} d^{-1}. Can data

from the present study close this imbalance further?

As we have seen, the ZOOFLUX technique in this instance was not able to provide an accurate assessment of the overall active flux for a variety of reasons. However, the data can still be used to estimate the maximum potential flux for comparison with previous estimates. For an average 3.5 mm-long *P. xiphias* adult, the mean carbon weight around dawn was 176 μg ind.$^{-1}$. For an average 10 mm-long krill, the mean carbon weight around dawn was 490 μg ind.$^{-1}$. The maximum migrating biomass (August 2000) was estimated at 42 *P. xiphias* m^{-2} d^{-1}, and 18 krill m^{-2} d^{-1}. If we assume that 10 % of the carbon weight will be lost at depth via defaecation, excretion and respiration, then for *P. xiphias* and krill combined, the active flux of carbon (as POC, DOC and DIC) would have been 1.6 mg C m^{-2} d^{-1}. This is quite evidently lower than previous estimates, suggesting that, at this time of year at least, the behaviour of the zooplankton community would not have been causing a significant export of carbon at BATS, and would therefore not have helped significantly to reduce the discrepancy in the carbon budget. Even accounting for the possibility that individuals may have contained as much as 25 % more carbon due to processing losses (see section 5.3.4), we can only increase this estimate to 2.0 mg C m^{-2} d^{-1}. It is evident that the magnitude of the migrating biomass will be a significant factor in the importance of the active flux relative to other fluxes, and that the calculations presented here include neither the other species of migrant to be found at BATS, nor an assessment of the active flux due to mortality and moulting at depth. However, it is highly unlikely that these considerations could boost the active flux to the required 96 mg C m^{-2} d^{-1}, suggesting that, while zooplankton DVM is certainly a component of the biological pump worthy of recognition in global carbon budgets, it is by no means the most important.

5.4.5 Summary: the active flux at BATS

1. Interzonal DVM was a feature in both sampling months (August and September 2000), with 4 times more biomass migrating in August.
2. It was estimated that between 12 and 42 individual *P. xiphias* per m^{-2} were migrating out of the top 200 m on a daily basis, and between 8 and 18 individual krill per m^2.
3. Both *P. xiphias* and krill appeared to perform a dawn descent and a dusk descent (NDVM). *P. xiphias* appeared to initiate the dawn descent earlier than krill.
4. The biometric data yielded by the onboard processing method were different to those yielded by the ashore method: gut fullness was 15-40 % higher in shore-processed individuals, while carbon and nitrogen weights were lower by 7-25 %.
5. For *P. xiphias*, defaecation did not represent a significant avenue for carbon and nitrogen loss relative to the mass of an individual.
6. The body condition (length:weight) of *P. xiphias* was found to be variable, and that of krill to be more uniform, during the present study.
7. For *P. xiphias*, there was a change in the size-structure of the population from July to September 2000, with the length-at-maturity becoming shorter over time.
8. *P. xiphias* collected in the field at different times during the diel cycle showed evidence for a diel feeding rhythm, feeding at higher levels in the top 200 m during the hours of darkness, and lower levels at depth during the day.
9. *P. xiphias* starved in laboratory incubations voided their guts within ~500 min, but no systematic decreases in body carbon or nitrogen weight were detected.
10. For *P. xiphias* and krill collected in the field at different times during the diel cycle, δ was found to be variable and V relatively high, while n was relatively low. The ability to measure the active flux at this site using ZOOFLUX would be improved with a better knowledge of the behavioural ecology of the migrating community.

6

DIEL VERTICAL MIGRATION IN A UNIQUE ENVIRONMENT: *NYCTIPHANES AUSTRALIS* (CRUSTACEA: EUPHAUSIACEA) IN DOUBTFUL SOUND, NEW ZEALAND

6.1 Introduction

6.1.1 An historical perspective

With the exception of Antarctica, New Zealand was the last large landmass to be colonised by humans. The original inhabitants were the Maoris, impressive ocean navigators who arrived from the Society Islands and/or the Cook Islands around 1000 AD. Genetic evidence has suggested that the Maoris' ancestors came from China, migrating through Taiwan, the Philippines, Indonesia and Polynesia before settling in New Zealand (e.g. Underhill *et al.*, 2001). Moreover, archaeological evidence has suggested that humans were transient through New Zealand ~1000 y prior to permanent settlement. The Dutch explorers Abel Tasman and Frans Visscher must have been among the first westerners to reach New Zealand, when they approached South Island in 1642. However, the most comprehensive primary accounts of "Aotearoa", the "land of the long white cloud", came from the famous 18th century English explorer Captain James Cook, who explored and charted both North Island and South Island on each of his three round-the-world voyages of discovery. In fact, so integral is Cook in New Zealand's relatively short human history that it is fitting to include a brief account here.

On his first Pacific voyage with the *Endeavour* (1768-1771), Cook sighted the east coast of North Island at 2 p.m. on the 7th October 1769. He first reached Fiordland, on the south-west coast of South Island, in March 1770. The following are extracts from his journal at this time (Edwards, 1999):

WEDNESDAY 14th. "At 2 oClock it clear'd up over the land which appear'd high and Mountainous. At half past 3 double reef'd the Topsails and haul'd in for a Bay wherein there appear'd to be good anchorage...but...we gave it up and bore away aLong shore. This Bay I have named *duskey Bay*..."

THURSDAY 15th. "A little before noon we pass'd a small narrow opening in the land where there appear'd to be a very snug harbour...It lies in the Latde of 45°16' s...The Land on each side of the entrance of this harbour riseth almost perpendicular from the Sea to a very considerable height and this was the reason why I did not attempt to go in with the Ship because I saw clearly that no winds could b(l)ow there but what was either right in or right out. This is Westerly or Easterly, and it certainly would have been highly imprudent in me to have put into a place where we could not have got out but with a wind that we have lately found does not blow one day in a month: I mention this because there were some on board who wanted me to harbour at any rate without in the least considering either the present or future consequences."

The "some on board" that Cook refers to were the ship's naturalists, led by Joseph Banks, a wealthy British landowner. As the story goes, the decision not to enter any of the Fiordland "bays" caused a heated argument to take place between Cook and Banks. Banks' journal mentions his frustration about sailing past apparently good harbours which, he says, would have allowed him "to examine the mineral appearances". It was thought that this argument occurred at the entrance to Doubtful Sound (or, as Cook called it, "*Doubtfull Harbour*"). This may well be why this fiord is so called, reflecting Cook's doubt as to its suitability as a harbour.

After Cook's visit, most of the early European exploration of Fiordland was carried out by sealers and whalers in the early 1800s (Glasby, 1978). This is not to forget, of course, that Fiordland also has an extensive Maori history (Beattie, 1949). Given the eclectic mix of primary visitors and inhabitants in this region, the origins of many of the named features remain unconfirmed, although interesting stories abound (see Glasby, 1978 for references). The first detailed survey of the Fiordland coastline was carried out from 1850 to 1852 in the Admiralty vessel "Acheron", from which British Admiralty Chart 768 was produced, and from which the Hydrographic Office chart NZ7522 (1959) was strongly based.

6.1.2 A history of research in Fiordland

Given its remoteness, it is perhaps not surprising that Fiordland (Figure 6.1) has received relatively little attention from the scientific community. Analysis of *Web of Science* entries for the period 1980-2002 revealed that there have been approximately 200 publications describing a variety of terrestrial and marine studies in Fiordland, and there appear to have been relatively few publications prior to this. Terrestrial studies have investigated topics ranging from geology and earthquakes (e.g. Davey & Broadbent, 1980; Smith & Davey, 1984; Reyners & Webb, 2002), to geochemistry (Pirajno, 1981), botany (e.g. Morrison, 1982; Allen *et al.*, 1994), bats (e.g. Parsons *et al.*, 1997; Sedgeley & O'Donnell, 1999), parakeets (Elliot *et al.*, 1996), mohua (a small, insectivorous bird: Elliot, 1996), kakapo (a large, flightless parrot: Powlesland & Lloyd, 1994), takahe (a flightless rail: Mills *et al.*, 1991), stoats (e.g. King, 1991; Dilks *et al.*, 1996), deer (e.g. Mark & Baylis, 1982; Nugent & Sweetapple, 1989), house mice (Ruscoe, 2001) and penguins (e.g. Grau, 1982; McLean *et al.*, 2000). Studies have also been carried out on the effects of tourism in the area (Booth, 1999; Hunt, 1999), while Mark (1998) reviewed the research that has justified the establishment of the 2.6 million ha south-west New Zealand World Heritage Area, of which Fiordland National Park is a part.

A large proportion of the marine studies in Fiordland appear to have been undertaken in Doubtful Sound, probably due, at least in part, to its accessibility via a mountain-pass road (Wilmot Pass) and the presence in Deep Cove of a small hostel and field laboratory. Scientists from the University of Otago in Dunedin have undertaken a large part of this research effort. The marine studies have investigated aspects of the ecosystem ranging from general hydrography (e.g. Batham, 1965; Stanton & Pickard, 1981; Gibbs *et al.*, 2000; Gibbs, 2001; Peake *et al.*, 2001), to rocky shore communities

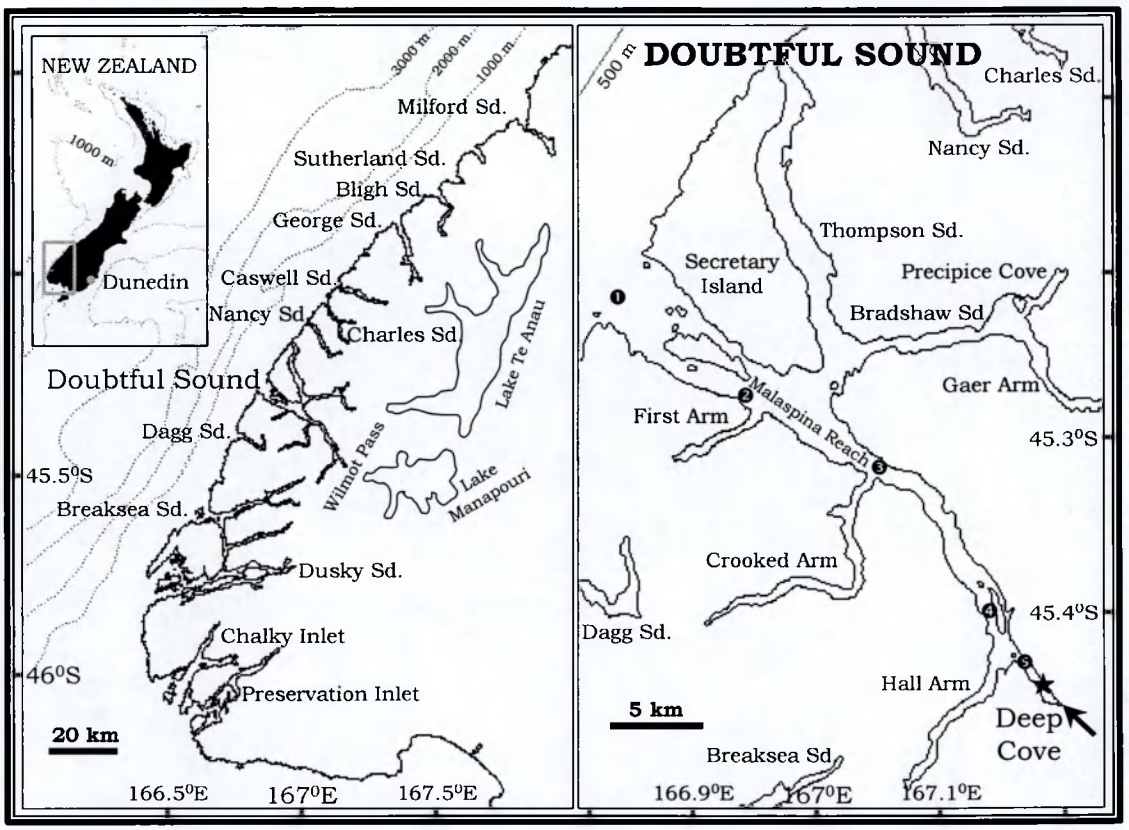

Figure 6.1 The location of Doubtful Sound in Fiordland National Park on the remote south-west coast of South Island, New Zealand. ★ shows the approximate location of sampling in Deep Cove during the present study. The arrow shows the point at which the Manapouri hydro-electric facility 'tailrace' flows into Deep Cove. The numbered circles represent some of the oceanographic stations previously investigated by Stanton & Pickard (1981). Water depths at these stations are as follows: (1) = 120 m, (2) = 420 m, (3) = 350 m, (4) = 140 m, (5) = 130 m.

(e.g. Batham, 1965; Witman & Grange, 1998; Smith, 2001), brachiopods (Campbell & Fleming, 1981; Chuang, 1994), sponges (Miller *et al.*, 2001), bryozoa (Smith *et al.*, 2001), rock lobsters (Annala & Bycroft, 1988, 1993), brittle stars (e.g. Stewart & Mladenov, 1995, 1997; Stewart, 1996, 1998), sea cucumbers, black coral (e.g. Miller, 1997, 1998; Parker *et al.*, 1997), bivalves (Marshall, 1998), sea urchins (e.g. Mladenov *et al.*, 1997; Brewin *et al.*, 2000; Lamare & Barker, 2001; Villouta *et al.*, 2001), zooplankton (Jillett & Mitchell, 1973; Hays *et al.*, 1998), and dolphins (e.g. Williams *et al.*, 1993; Brager & Schneider, 1998; Haase & Schneider, 2001).

Doubtful Sound has a unique marine ecosystem, with a low abundance of macrophytes and a dominance by sessile suspension and filter feeders. Furthermore, the particular bathymetry and hydrography of this fiord allows certain organisms to grow here at depths much shallower than they would normally be found. These organisms include black coral (*Antipathes fiordensis*), red coral (*Erina novaezelandia*), snake stars (*Astrobrachion constrictum*) and brachiopods (e.g. *Liotherella novaezelandia*). For SCUBA divers, this poses a unique opportunity to see a variety of deeper-water species first-hand which would normally be beyond the range of compressed-air dives.

6.1.3 Fiordland hydrography

From her study of the rocky shore ecology of a southern New Zealand fiord, Batham (1965) provided what is probably the first description of the hydrography in Doubtful Sound. At the time of sampling (February 1957), the Lake Manapouri hydro-electric facility and accompanying road had not yet been built, and Deep Cove was accessible only via a 10-mile "tramping" track. Since this study, there have been approximately twelve publications which describe the hydrography of this and other fiords in the region (All fiords: Stanton and Pickard, 1981. Milford Sound: Garner, 1964. Caswell Sound: Stanton, 1978. Nancy Sound: Stanton, 1978. Dusky Sound: Jillett & Mitchell, 1973. Doubtful Sound: Stanton, 1986; Davies-Colley, 1992; McCully *et al.*, 1995; Witman & Grange, 1998; Bowman *et al.*, 1999; Gibbs *et al.*, 2000; Gibbs, 2001; Peake *et al.*, 2001). The descriptions below concentrate mainly on the hydrography of Doubtful Sound.

From Milford Sound in the north, to Preservation Inlet in the south, the various inlets of Fiordland span ~200 km of coastline. These steep-sided, deep inlets are the drowned lower reaches of valleys formed ~15,000 years ago by glaciation (Cotton, 1956). The

mountainous topography of the region, with peaks reaching 2000 m or more, and the exposure to the prevailing westerly weather systems, results in high levels of rainfall with maxima in November and March, and minima in July and January (Stanton & Pickard, 1981). Annual normal rainfall in Deep Cove is 5290 mm, while in nearby Wilmot Pass (only ~4 km away) it is 6747 mm (Stanton & Pickard, 1981), demonstrating the marked effects of topography. Similarly, the large catchment area of Doubtful Sound (whole fiord: 627 km^2; Deep Cove: 69.8 km^2) compared to its surface area (whole fiord: 83.7 km^2) results in high levels of freshwater input from September through to February (Stanton & Pickard, 1981). Fluvial input to the whole fiord averages 135 m^3 s^{-1} (Bowman *et al.*, 1999). This freshwater inflow is significantly augmented by the 600 megawatt Manapouri hydro-electric power station, which routes water from alpine Lakes Te Anau and Manapouri, 10 km through the mountains beneath Wilmot Pass and into Deep Cove. This inflow averages 450 m^3 s^{-1} (Gibbs *et al.*, 2000), making it the most significant source of freshwater in the region, and making Doubtful Sound unique in this respect in Fiordland (cf. 15-40 m^3 s^{-1} from the Cleddau River flowing into Milford Sound: see Appendix 1 in Stanton & Pickard, 1981). All fiords in the region exhibit typical estuarine circulation: the seaward flow of a surface low-salinity-layer (LSL) is compensated for by a deeper inflow of coastal-derived seawater.

Temperature and salinity profiles from Deep Cove in December 1977 revealed a cool LSL extending from the surface to ~5 m depth, below which temperature and salinity remained relatively constant (Stanton & Pickard, 1981). It is therefore apparent that, apart from the strong gradient between the surface LSL and the underlying layer, there was no marked pycnocline in Deep Cove. Stanton & Pickard (1981) maintained that the sills in Doubtful Sound are deep enough to allow a relatively free flow of deep water between basins. The deepest basin in Doubtful Sound, in Malaspina Reach, reaches 465

m. Indeed, this free-flow of deep water is true for all of the fiords in this region, for which reason anoxia is not a feature of the deep-water masses. However, Stanton & Pickard (1981) also showed that there may be some degree of inhibition of deep-water flow at the heads of several fiords, especially the longer ones such as Doubtful Sound and Milford Sound, where inner basin sill depths are shallower than outer basin sill depths (e.g. Doubtful Sound: outer sill = 101 m; Deep Cove sill = 55 m).

Gibbs *et al.* (2000) described and modelled the structure, variability and dynamics of the LSL in Doubtful Sound, partly as a context to ecological studies such as those of Witman and Grange (1998) on the rock-wall assemblages. They showed that the LSL in Deep Cove is robust and exaggerated due to the hydro-electric facility, and that it is affected by wind-stress and rainfall. Strong, persistent westerly winds were found to cause a "storm surge" in Deep Cove, whereby the LSL "piles up" and deepens towards the head. This storm surge is able to slow, or even reverse the estuarine circulation. The depth of the LSL is therefore subject to variability in line with both wind-stress and precipitation, and may be as much as 12 m thick on occasion. Salinities in the LSL typically range from 5 to 10 psu. As discussed in Gibbs (2001), the LSL has an important influence on the sub-surface light field, and therefore may also prove to be of importance in the ecology of the zooplankton community.

Light levels in Doubtful Sound are also affected by the presence of high levels of "yellow substance" in the water, as described by Davies-Colley (1992). They found that the concentration of this yellow substance, most of which is "old material, derived from terrestrial production or long past marine production", was more than two orders of magnitude higher in the low-salinity surface waters of Doubtful Sound than in the cold subantarctic water to the south-east of South Island, resulting in particularly high levels of light absorption.

6.1.4 Fiordland zooplankton

Past studies

There appear to have been only two publications concerning the zooplankton in Fiordland. To commemorate the bi-centenary of Captain James Cook's first visit to New Zealand, Jillett & Mitchell (1973) conducted a 10-day survey of temperature, salinity, dissolved oxygen, chlorophyll *a* and zooplankton in Dusky Sound in February 1969. Oblique net tows from 50 m and 100 m to the surface were made with a 132 μm-mesh net at nine stations within the fiord system. At two of these stations, tows were also made from just below the surface, and just below the LSL. The zooplankton community was dominated by species with neritic affinities, including the copepods *Paracalanus parvus*, *Acartia clausi* and *Oithona* spp., the cladocerans *Evadne* sp. and *Podon* sp., barnacle nauplii and the meroplanktonic larvae of various benthic and littoral species. It was deemed "unusual", however, that a variety of species with open-water affinities were also found, even at the heads of the inlets. These included the copepods *Ctenocalanus vanus*, *Metridia lucens*, *Candacia* sp. and *Oncaea* sp., the krill *Nyctiphanes australis*, and a variety of other, less abundant species. The higher concentration of zooplankton at the heads of the inlets was attributed to the estuarine pattern of circulation, whereby animals are carried up-fiord in the inflowing deep-water and gradually concentrated at the head as they avoid the entrainment of the outflowing surface LSL. While certain species were described as being "confined" to either the deep water (e.g. *C. vanus*, *N. australis*) or the surface (e.g. *A. clausi*), there was no mention of VM behaviour despite the presence of known migrants such as *M. lucens* and *N. australis*. Finally, it was shown that the zooplankton community was similar at all stations within the fiord system, differing markedly only at the mouth as the influence of coastal water became more pronounced.

The second zooplankton study was undertaken in September 1996 at a station in Deep Cove, Doubtful Sound (Hays *et al.*, 1998). Unlike the mainly descriptive study by Jillett & Mitchell (1973), this study addressed more of an ecological theme. It was shown that the known vertical migrant *M. lucens* exhibited DVM behaviour in Deep Cove, but that ~40 % of the population remained near the surface during the day. From observations of a diel change in the carbon and nitrogen weight of individuals at the surface, but not in their prosome length, it was inferred that daytime avoidance of the surface layer was only taking place by individuals with a better body condition (i.e. a higher carbon or nitrogen content per unit length). While no other physical or biological parameters were measured, it was suggested that DVM behaviour by *M. lucens* in Deep Cove was a function of the classic trade-off between eating and being eaten: individuals with a lower body condition needed to remain in the surface layer, even during the day when the risk of predation was high, in order to build up body reserves for successful reproduction. This explanation is therefore based on the assumption that both food levels and predation pressure are higher towards the surface at this site. If this is indeed the case, the presence of DVM and a stratified food regime are indicative that an active flux may be occurring, forming the reason why this site was deemed suitable for the third and final field-test of ZOOFLUX by the present study.

Nyctiphanes australis

The euphausiid crustacean *Nyctiphanes australis* (Sars, 1883) was collected in Deep Cove and analysed during the present study. *N. australis* is epipelagic, and is found in coastal waters off New Zealand and south-east Australia (latitudinal range 35-50 °S) (Mauchline & Fisher, 1969). These environments typically encompass temperatures >13.5 °C, and salinities ranging from 34.05 to 34.72 psu. Sheard (1953) described the

development of *N. australis* in some detail, showing that the life cycle typically includes two naupliar stages, a metanauplius (the hatching stage), three calyptopis stages, and three furcilia stages (followed by sub-adults and then adults). Adults are >11 mm in length (Sheard, 1953), and may reach up to ~20 mm (e.g. Ritz & Hosie, 1982).

According to Sheard (1953), *N. australis* is omnivorous, eating detritus, diatoms and crustacea. Dalley & McClatchie (1989) showed that, while the feeding basket is highly setose and the internal armature of the stomach is heavily spinose (suggesting herbivory), the mandibles resemble those of predominantly carnivorous species such as *Meganyctiphanes norvegica*. They therefore concluded, as did Ritz *et al.* (1990), that this species is an opportunistic omnivore, although confirmation from stomach contents could not be satisfactorily provided in the former study. Pilditch & McClatchie (1994) demonstrated that *N. australis* preferred the copepod *Acartia* spp. over the diatom *Chaetoceros gracilis*. However, the rate of ingestion of *Acartia* was significantly reduced in the presence of *C. gracilis*.

In turn, *N. australis* is eaten by a variety of predators. Fish predators include southern bluefin tuna, striped tuna, skipjack tuna, jack mackerel, barracouta, Australian pilchard and tiger flathead off Australia (Sheard, 1953; Ritz & Hosie, 1982), and barracouta off New Zealand (Blackburn, 1957). They are also predated on by squid off New Zealand (Mauchline, 1980). Avian predators include the Tasmanian mutton bird (Sheard, 1953; Morgan & Ritz, 1982), grey faced petrels and fairy prions (Bartle, 1976; Morgan & Ritz, 1982). Due to its pivotal role in the food chain in these regions, *N. australis* is therefore an important species within the marine economy (Dakin & Colefax, 1940; Sheard, 1953; Blackburn, 1980).

Like most krill, *N. australis* is known to undertake DVM (e.g. Bary, 1956). It has been found to reside between 100 and 200 m by day, between the surface and 100 m at

night, and never to exceed a depth of 400 m at any time (Mauchline & Fisher, 1969; Bartle, 1976). However, from samples collected off Tasmania, Young *et al.* (1993) stated that this species "does not regularly migrate vertically". Surface swarms and "surface rafts" have been observed on many occasions, both before and during the breeding season (Sheard, 1953; Komaki, 1967; Bradford, 1972; Kawakami *et al.*, 1973). As Mauchline (1980) pointed out, the "presence of such aggregations seriously affects the interpretation of quantitative samples taken to assess the vertical distributions and migrations of species". These swarms might also be expected to be susceptible to predation from whales, but no records have been provided to date.

Sheard (1953) showed that *N. australis* had a protracted breeding season of 8-11 months, while Taw & Ritz (1979) showed that breeding was continuous. As Hosie & Ritz (1983) stated, "*N. australis* appears unique amongst euphausiids...in having continuous maturation of the ovaries", while their observation of a very small proportion of females with spent ovaries suggested that "the ovaries do occasionally regress to a resting stage". Applying growth curves to their longest specimen caught, Ritz & Hosie (1982) estimated that individuals are unlikely to live longer than a year. Hosie & Ritz (1983) showed that larvae moulted approximately every 2.5 to 3 d and adults every 4 to 5 d at 15 °C, and that the intermoult period was nearly doubled at 10 °C. Exuviae represent ~5-10 % of the total body dry weight, such that moulting forms a significant contribution of organic material to the environment (Lasker, 1964; Ritz & Hosie, 1982; Hosie & Ritz, 1983). It must be borne in mind, however, that these studies were carried out in Australia, and that there may be regional differences in growth and reproductive activity.

6.2 Materials and methods

6.2.1 Sampling site and schedule

Sampling was carried out in Deep Cove at the head of Doubtful Sound (Figure 6.1) during the austral summer of 2000/2001. Doubtful Sound, at ~40 km long, is the largest of the various fiord systems which together make up the Fiordland National Park on the remote south-west coast of South Island, New Zealand The exact site visited (45.45 °S, 167.15 °E) was located opposite Lady Alice Falls in the middle of Deep Cove, where the water depth was 125 m as measured by the onboard echosounder. This site was chosen for four main reasons: (1) the water depth here meant that there was a strong possibility of finding vertically migrating zooplankton; (2) the prominent landmark of Lady Alice Falls provided a quick visual reference-point for the site, a useful feature given that the surface current would often cause considerable westward drift during tows, necessitating a return before the next net deployment. This was also useful in locating the site quickly at night, the light colour of the falling water being visible even on cloudy nights; (3) after samples had been collected, it took less than 5 min to return to shore, allowing for sample processing to be completed within 40 min of collection; (4) this was the exact site of a previous zooplankton study (Hays *et al.*, 1998), and so might allow direct comparisons of the zooplankton collected in both studies to be made. Sampling was conducted from a 5.8 m rigid hulled inflatable "Naiad" operated by the Portobello Marine Lab (PML), University of Otago, Dunedin. Deep Cove was visited twice for this study, from November 28th to December 1st 2000, and from December 10th to 13th 2000.

6.2.2 Temperature and salinity measurements

Profiles of water temperature and salinity were obtained at 1 m intervals from the

surface to 9 m using a hand-held water meter (Horiba Water Checker U-10). The maximum depth of these profiles was constrained by the length of the cable. A weight was attached near to the end of the cable to ensure that the sensor descended vertically in the surface current.

6.2.3 Light measurements

Solar radiation was measured with a hand-held light meter. Readings were taken as often as possible during the dawn and dusk periods. The sensor was housed in a water-tight unit resembling a light bulb, and so was also used to measure light levels through the water column. The maximum depth of these profiles was constrained by the length of the cable (9 m).

6.2.4 WP-2 net tows

Zooplankton were collected using a 1 m diameter, 500 μm-mesh WP-2 net (Unesco, 1968) with filtering cod-end which could be opened and closed at discrete depths with the use of an OTE double-trip release mechanism. This was the same net as that used in the Clyde Sea (see section 4.2.5). On both visits to Fiordland, sampling was undertaken at dawn and dusk, as well as at one or more time-points during the day and night (Figure 6.2). With the boat stationary, the net was hauled vertically (at approximately 0.5 m s^{-1}) via a hydraulically-powered custom-built winch based on the design of a crayfish-pot winch. The cable used was 6 mm rope with kevlar braid to prevent stretch. Coded markings at 10 m intervals allowed the depth of the tows to be assessed.

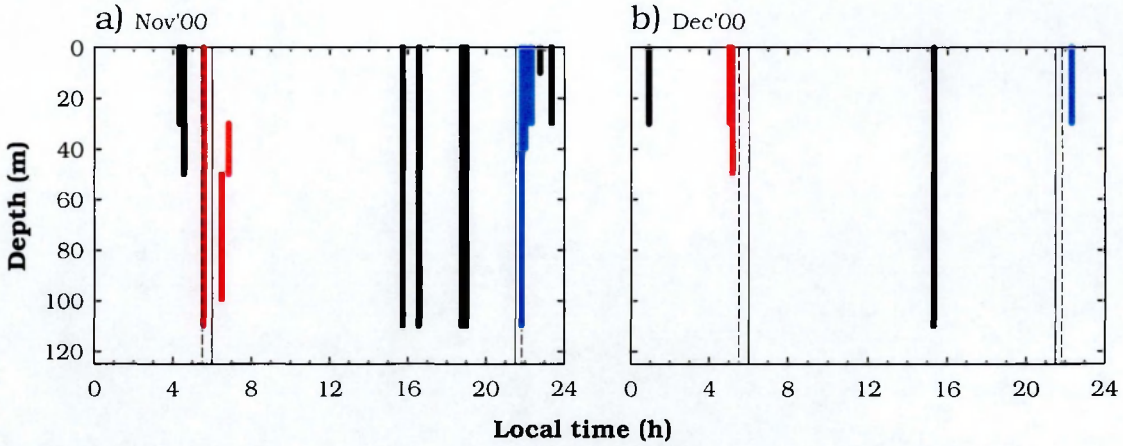

Figure 6.2 The time and depth of WP-2 net tows made in Deep Cove for the collection of *Nyctiphanes australis*. The red and blue lines represent 'dawn' and 'dusk' tows, respectively, and the thick black lines represent tows made at other times. The broken and solid vertical lines show the timings of first/last light and sunrise/sunset, respectively.

During the first visit, exploratory tows were made to ascertain the whereabouts of any potential migrators at any given time. The contents of these tows were poured gently into labelled 1000 ml clear plastic screw-top pots, and a plastic wash-bottle containing surface fiord-water used to ensure that all animals were washed out of the cod-end. An immediate visual inspection of the pots gave a rough idea of the vertical distribution of the larger zooplankton and the likely whereabouts of any potential migrants. Based on this information, a final tow was made at an appropriate depth in order to collect migrants for biometric measurements. This sample was decanted into a separate 1000 ml clear-plastic screw-top pot. This pot contained ~50 ml of soda water in order to anaesthetise the animals. However, after a few tows, it was apparent that animals were coming up either moribund or dead, possibly due to being brought up through the pronounced surface freshwater layer, and so the use of soda water was deemed unnecessary. The boat returned to shore within 5 min of the final sample collection, and the samples were taken to a field station for immediate processing.

During the second visit, a more uniform series of tows was made. At any given time,

a tow was made at each of four depth increments within the 125 m water column: 0-30 m, 30-50 m, 50-70 m and 70-100 m. These samples were destined for fixation, preservation and future identification and enumeration. A fifth tow was made in order to collect migrants for biometric measurements. As before, the appropriate depth for this tow was estimated from a visual inspection of the preceding tows. Unlike before, animals were coming up still alive, possibly due to the presence of a less-pronounced surface freshwater layer at this time (freshwater input was reduced due to the cessation of the hydro-electric facility "tailrace" between December 2nd and 18th), so soda water was used on this occasion to anaesthetise them.

Sample processing

Upon returning to shore and securing the boat, the samples were taken into the field station. Starting with the most recent tow, which was designed to collect migrators from their estimated depth at that time, the contents of the plastic pot were poured gently through a 5 cm-diameter 500 μm-mesh sieve, which was placed in a petri dish containing just enough water to cover the bottom of the mesh. Under a Nikon binocular microscope, individual *Nyctiphanes australis* were measured for length (middle of eye to end of telson) using an eyepiece graticule precise to ±0.14 mm. In addition, an estimate of individual gut fullness was made, as the gut contents were clearly visible through the semi-transparent body wall (this estimate was based on the relative length of the gut contents, and expressed on a scale of 0 to 100 by 5 % increments). For consistency, these measurements were carried out by myself throughout. After measuring, individuals were transferred to individual wells in a clean 96-well microtitre plate.

The microtitre plates were labelled and immediately placed in a portable electric

food desiccator (essentially a small fan-assisted oven) with their lids removed. The samples were dried in this manner at 60 °C for ~48 h, after which the lids were replaced and the boxes transferred to an airtight sandwich box containing self-indicating desiccant crystals, ready for transport back to the home laboratory in Dunedin (PML). For those samples processed <48 h before leaving the field, the semi-dried boxes were also transported in the sandwich box, but returned to the desiccator for the requisite amount of time when a power source became available.

The remaining tows were poured in turn through the 5 cm-diameter 500 μm-mesh sieve, and the stranded animals transferred to 500 ml plastic screw-top jars using a plastic wash-bottle containing fresh tap water. Tap water was added to each jar so that they were just under 90 % full, and a solution of 40 % buffered formalin (~15 % formaldehyde) added to nearly fill each jar. This resulted in a fixative solution of ~4 % formalin, (= ~1.5 % formaldehyde). The buffer used was 30 g of sodium tetraborate (borax) per litre of 40 % formalin. The samples were maintained in this fixative for a minimum of 2 months before being transferred into 90 % alcohol for long-term storage.

All dried samples were weighed on a Sartorius "Micro" electrobalance precise to ±1 μg, having been left in the sandwich box for at least 24 h in the same room as the balance. This allowed the samples to acclimate to the ambient room temperature, so reducing their strongly hygroscopic nature which can cause significant problems when making sensitive mass measurements. Following weighing, a proportion of the samples were placed into individual Elemental Microanalysis Ltd. tin capsules (8 mm × 5 mm), and their total carbon and nitrogen weights measured with a Carlo Erba elemental (CHN) analyser (precise to ±0.01 μg) located in the Department of Chemistry, University of Otago.

Each of the preserved samples in turn was drained through a 100 μm-mesh sieve and

transferred to a Folsom plankton splitter containing ~500 ml of tap water. The number of splits made depended on the amount of zooplankton in the sample. The final subsample was decanted into a petri-dish, on the underside of which had been scored a grid of small squares. All specimens within the subsample were identified and counted under a binocular microscope (Olympus SZX 9 with 10× magnification eyepieces). For the more common taxa, as many as 484 individuals were counted per subsample. The various calanoid copepod taxa were matched as closely as possible in terms of gross morphology and size to the descriptions provided by Bradford-Grieve (1994, 1999). It was deemed unnecessary for the purposes of this study to confirm the species identity of every specimen, an exercise which would have required much more detailed morphological analyses and expertise in many cases. The calanoid taxa identified refer to predominantly adult specimens, while an "unidentified copepodites" category was generated to include the juvenile stages of these taxa as well as the juvenile and adult stages of other, unidentified species. While crustacean nauplii were present in many of the tows, they were not counted here.

6.3 Results

6.3.1 The physical environment: temperature, salinity and light data

Profiles of temperature and salinity

The Manapouri hydro-electric power-station 'tailrace' (see Figure 6.1), which provides by far the greatest input of freshwater into Deep Cove, was switched off from December 2nd to 18th 2000 for the final drilling stages of a second turbine tunnel beneath Wilmot Pass. This meant that the tailrace was not operative during the second sampling visit of the present study. The temperature and salinity profiles to 9 m from the first visit (November 2000) and the second visit (December 2000) are shown in Figure 6.3

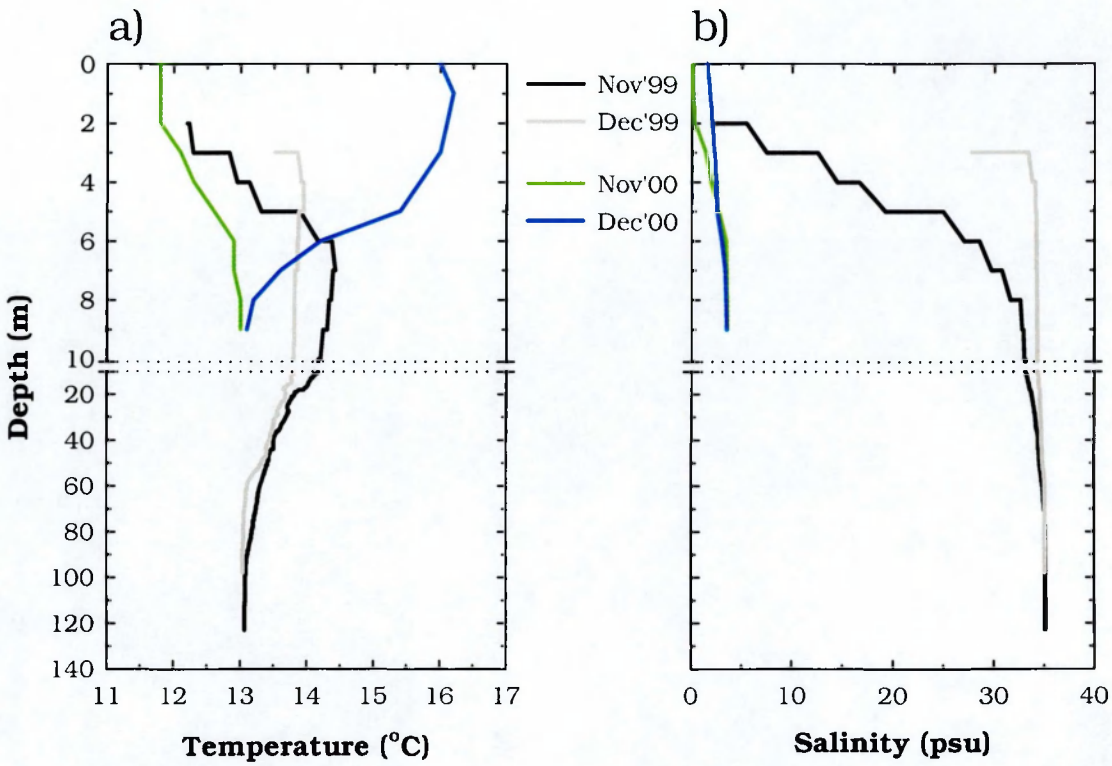

Figure 6.3 Hand-held water-meter (blue and green lines: November and December 2000) and CTD-measured (black and grey lines: November and December 1999) profiles of a) temperature, and b) salinity in Deep Cove. Note the changing scale of the *Y*-axis to highlight better the changes occurring in the top 10 m. Processed CTD data for 1999 courtesy of P. Brewin, University of Otago.

alongside deep profiles (to 120 m) measured with a SeaBird SBE-19 CTD in November and December 1999 (courtesy of P. Brewin). The deep profiles from the previous year show that both temperature and salinity were low in the top 10 m at this time, although there were noticeable differences between months. In November 1999, the surface low-salinity-layer (LSL) extended to ~6 m, while in December 1999 it extended to ~3 m. Below this layer, the water mass was relatively homogeneous in terms of both temperature (mean = 13.5 °C) and salinity (mean = 35 psu). The salinity profiles in 2000 showed that the LSL extended to at least 9 m in both November and December, with values increasing gradually from <2 psu at the surface to ~4 psu at 9 m. While the water in the top 4 m was fresher in November 2000 (0 psu versus 2 psu), the LSL was

still noticeable in December 2000. The surface temperature was ~4 °C warmer in December 2000 (16 °C) than in November 2000 (12 °C). By 9 m, temperatures were the same in both months (~13 °C).

Light

Sunrise was at 06:00 h and sunset at 21:30 h during this study (data from URL: http://aa.usno.navy.mil/data/). Figure 6.4a shows the light levels around these times, as measured in November and December 2000. In both months, first light occurred ~30 min before sunrise, and last light ~20 min after sunset. While levels of light attenuation in the water column were extremely high in both months, they were noticeably higher in December than in November (Figure 6.4b). Within the top few cm, the ambient light levels had been reduced by 70 % in November, and 85 % in December. By 9 m, the ambient light levels had been reduced by 94 % and 98 %, respectively.

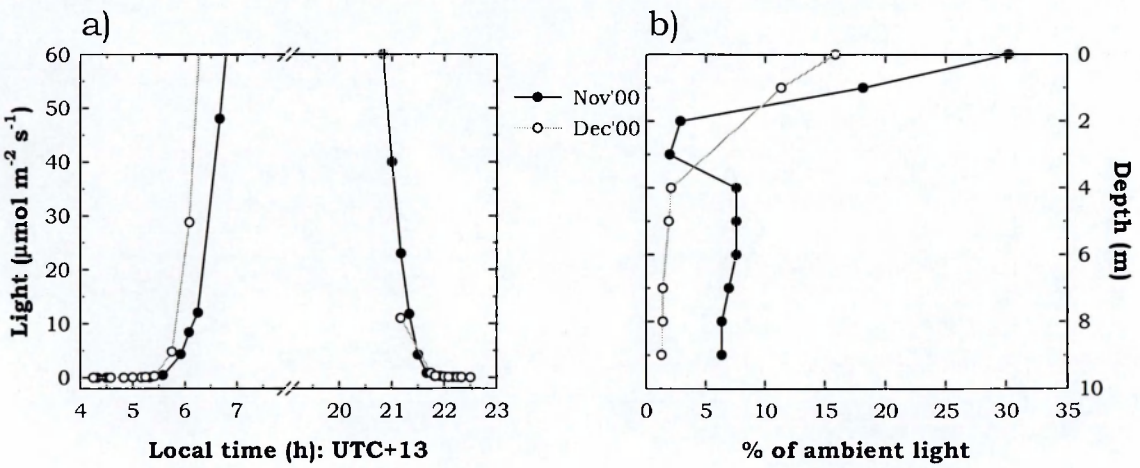

Figure 6.4 a) Light levels around dawn and dusk in Deep Cove in November and December 2000. b) Profiles of daytime light levels in the top 9 m of the water column in November and December 2000, expressed as a percentage of the ambient light levels at the time.

	Concentration (ind. m^{-2}, 0-100 m)			
	Nov'00 (night)	Nov'00(day)	Dec'00(night)	Dec'00 (day)
Copepods				
Acartia sp.	10	3	10	18
Calanoides sp.	0	0	30	3
Calanus australis	22	33	1683	1269
Candacia sp.	1	0	11	1
Centropages spp. (mostly *aucklandicus*)	4	11	85	98
Clausocalanus spp.	65	69	87	37
Egg sac copepod	0	0	21	37
Eucalanus spp.	0	1	53	90
Metridia lucens	281	37	107	10
Oithona spp.	10	537	92	141
Oncaea sp.	0	0	6	20
Paracalanus sp.	0	0	30	61
Rhincalanus nasutus	1	0	0	1
Unidentified copepodites	104	92	539	550
Krill				
Calyptopes	10	45	685	1120
Furciliae	8	6	203	177
Nyctiphanes australis sub-adults/ adults	295	8	339	137
Other				
Appendicularians	1	13	289	190
Barnacle nauplii	1	10	6	1
Chaetognaths	15	4	17	0
Cumaceans	0	0	1	0
Decapod larvae	103	43	32	43
Evadne sp./*Podon* sp. (cladocera)	28	0	435	592
Fish eggs	14	34	31	23
Fish larvae	3	4	36	15
Foraminifera	1	23	2	1
Hydromedusae	1	39	11	44
Hyperiids	5	1	8	1
Mysids	15	3	16	3
Ostracods	0	4	1	7
Polychaetes	1	22	5	9
Pteropods	0	0	1	42
Siphonophores	0	10	5	1
Stomatopods	0	0	0	1

Table 6.1 The total concentration of all net-caught mesozooplankton (>500 μm) in Deep Cove (ind. m^{-2}, 0-110 m) in November and December 2000.

6.3.2 Secondary production: mesozooplankton dynamics

Net-catch data

The mesozooplankton community: composition and concentration

Table 6.1 shows the composition and concentration (ind m^{-2}, 0-100 m) of all net-caught mesozooplankton (>500 μm) in November and December 2000. In both months, the zooplankton community consisted of both neritic and open-water components. In November, the dominant components were mostly open-water taxa, the most numerous of which were the krill *Nyctiphanes australis*, the copepod *Metridia lucens*, and a variety of decapod larvae. However, the neritic copepods *Oithona* spp. and *Clausocalanus* spp. were also numerous at this time. The remaining copepod fraction was not identified to species level. In December, the number of individuals was less evenly spread amongst taxa, with neritic taxa becoming more numerous, in particular the copepods *Oithona* spp., *Clausocalanus* spp., *Centropages* spp. and *Paracalanus* spp., and the cladocerans *Evadne* sp. and *Podon* sp.. Numerically abundant open-water taxa included *N. australis* (sub-adults/adults) and both calyptopes and furciliae, the copepods *Calanus australis*, *Metridia lucens* and "*Eucalanus* spp." (from the species and their morphological features shown in Bradford-Grieve, 1994, 1999, this is likely to have been a mixed group, consisting of *Subeucalanus* spp., *Pareucalanus* spp. and *Eucalanus* spp.), and appendicularians. Copepod specimens not identified to species level made up a larger fraction of the sample compared with November.

Many of the taxa present in November (both neritic and open-water) had increased in numbers by December, most notably *Calanus australis*, *Centropages* spp., *Eucalanus* spp., calyptopes, furciliae, appendicularians, cladocera and 'unidentified copepodites'. Conversely, the numbers of *Metridia lucens* showed a noticeable decrease. In the case of *Metridia lucens*, *Nyctiphanes australis* (sub-adults/adults) and mysids, there were

marked differences in the numbers of individuals caught by day and by night, with greater numbers being caught at night during both sampling visits.

The mesozooplankton community: vertical distribution

There were insufficient preserved samples available from the daytime in November to merit a graphical assessment of the day/night changes in vertical distribution. There were, however, sufficient samples from December, and these showed that the bulk of the zooplankton community remained in the surface layer (0-30 m) both night and day (Figure 6.5). Those taxa confined almost exclusively to the top 30 m included *Calanoides* sp., *Oncaea* sp., *Paracalanus* sp., 'unidentified copepodites', furciliae, chaetognaths, decapod larvae, cladocera and fish eggs, with only a small fraction (<~20 % of the population) being found deeper than this at any time.

A variety of other patterns were also apparent, although their definitions were blurred in many cases. Some taxa were spread more between the surface and 50 m, with only a small fraction (<~20 % of the population) being found deeper than this at any time. These included *Candacia* sp., *Oithona* spp., calyptopes and appendicularians. For a number of other taxa, while still found predominantly in the top 30 m both day and night, a noticeable fraction (up to 50 % of the population) was also found in the deeper-water depth intervals (often 70-100 m) at various times. These included *Acartia* sp., *Calanus australis*, *Centropages* spp., 'egg sac copepods', *Eucalanus* spp., *Metridia lucens*, *Nyctiphanes australis* (sub-adults/adults) and polychaetes. The remaining taxa exhibited a variety of patterns, some of which might suggest that DVM was taking place (*Clausocalanus* spp., barnacle larvae and mysids).

Of the zooplankton taxa identified during the present study, both *Metridia lucens* (adults) and *Nyctiphanes australis* (sub-adults/adults) are known, from studies in other

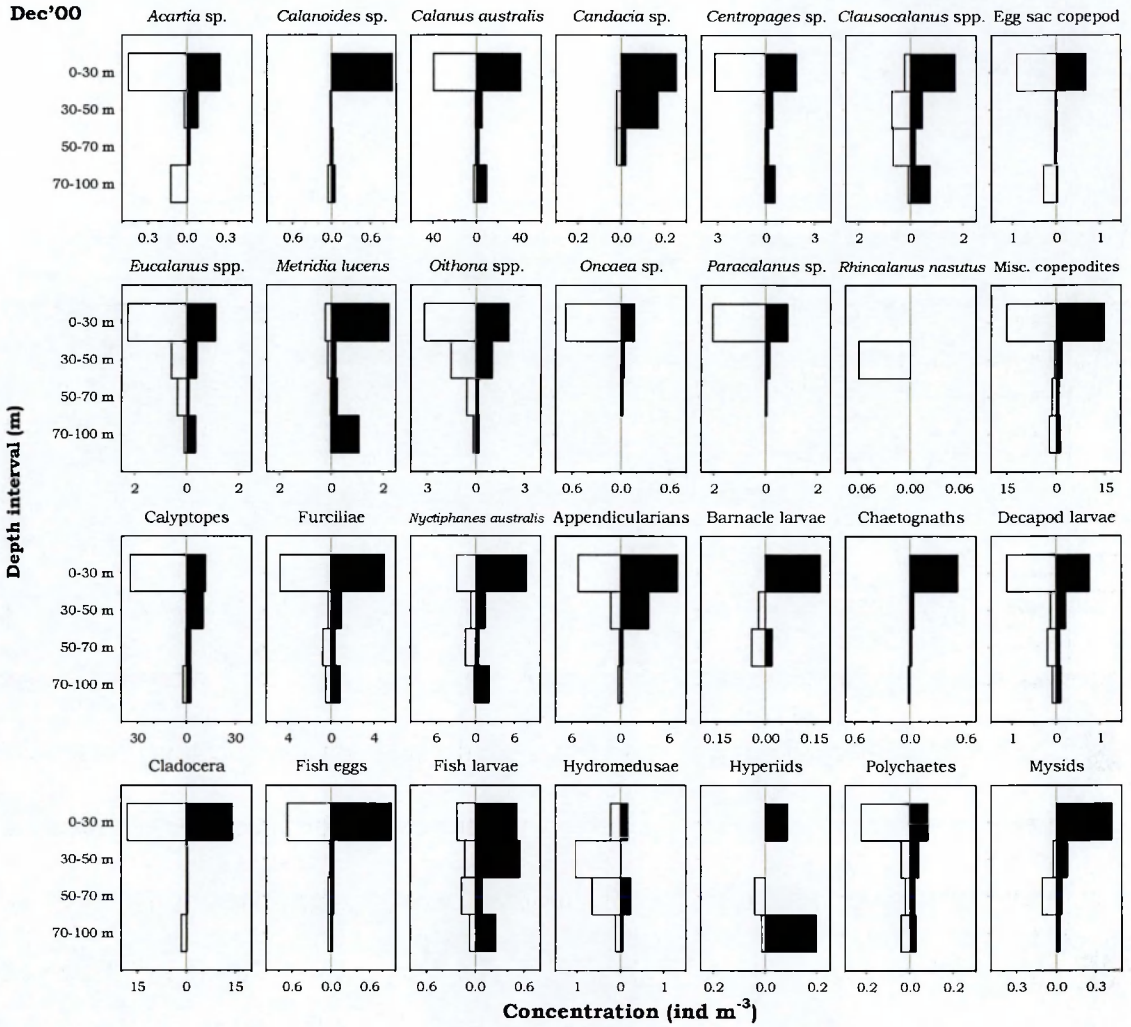

Figure 6.5 The vertical distribution and concentration of mesozooplankton (>500 μm) in Deep Cove by day (white bars) and by night (black bars) in December 2000. Note the changing *X*-axis (concentration) scale between each chart.

areas, to undertake significant DVM. The findings from the present study showed that both species had a similar nighttime pattern of vertical distribution: while the nighttime data from November are not graphed here, given that insufficient comparative daytime data were available, they closely approximated those from December as shown in Figure 6.5. While present at all depths from 0 to 100 m during the hours of darkness, both species showed much higher concentrations in the top 30 m, as well as a noticeable presence in the 70-100 m depth interval. This suggested that most of the population was

migrating into the top 30 m at night, but that a proportion also remained at depth at this time. The presence of a smaller proportion of *N. australis* at depth during the night in November (data not shown here) might suggest that a greater proportion of the population was migrating to the surface than in December. For *N. australis*, the concentration at the surface during the night in December reached ~8 ind m^{-3}. In November, these concentrations reached up to ~30 ind m^{-3} on occasion, although the numbers of individuals caught in the various nighttime net-tows varied markedly. The November tows also suggested that the bulk of the krill population was to be found in the 5-10 m depth interval at night, given high counts in 0-10 m tows and the complete absence of individuals in a tow to 5 m.

The lower daytime catches of both *N. australis* and *M. lucens* suggests that there was a daytime sampling artifact particular to these species at this location, which in turn reduced the ability to interpret accurately their vertical distribution and the possibility that they had been performing DVM. All that we can say from these data is that at least some of the population of both *M. lucens* and *N. australis* remained in the surface layer during the day.

			Individuals			
Visit	No. of net tows	Dev. stage	L values	GF values	DW values	C&N Values
Nov'00	15	Sub-adults/adults	178	171	178	60
	11	Furciliae	37	27	37	8
Dec'00	5	Sub-adults/adults	114	114	114	50
	5	Furciliae	124	124	124	0
			453	**436**	**453**	**118**

Table 6.2 The number of *Nyctiphanes australis* collected from WP-2 net tows in Deep Cove and measured for length (L), gut fullness (GF), dry weight (DW) and carbon and nitrogen weight (C & N).

Parameter	Precision	Data accuracy (%)	
		Sub-adults/ adults	Furciliae
Prosome length	± 0.14 mm	± 1.0 – 1.8	± 1.9 – 4.5
Dry weight	± 1 µg	± 0.03 – 0.3	± 0.2 – 3.1
Carbon & nitrogen weight	± 0.01 µg	± <0.1	± <0.1 – 0.3

Table 6.3 The precision and accuracy range of biometric measurements made on *Nyctiphanes australis* collected in Deep Cove in November and December 2000.

Biometric measurements of Nyctiphanes australis

Sample size and data quality

Table 6.2 summarises the number of samples collected and the number of biometric measurements made during each sampling visit. Table 6.3 shows the precision of the measuring instruments used, and the subsequent accuracy of the biometric data. By way of quality control, only those data values with an accuracy of ±5 % or less were deemed trustworthy enough for inclusion in further analyses (see Equation 2.2). The combination of relatively high instrument precision and the large size of individuals meant that all data were sufficiently accurate.

Relationships between parameters: investigating the ecology of individuals

Figure 6.6 shows that the body weight (dry, carbon or nitrogen weight) of *N. australis* increased exponentially with increases in body length (i.e. $Y = aX^b$). The log transformation of the X and Y values therefore allowed this relationship to be described in linear terms (i.e. $\log Y = \log a + b \log X$). When data from November and December were combined, the log length/log weight relationships were strong ($r^2 = 0.93$ to 0.95), and statistically significant (ANOVA: $P < 0.001$ in all cases). That is to say, changes in body length explained 93 to 95 % of the changes in body weight. The data deviated very

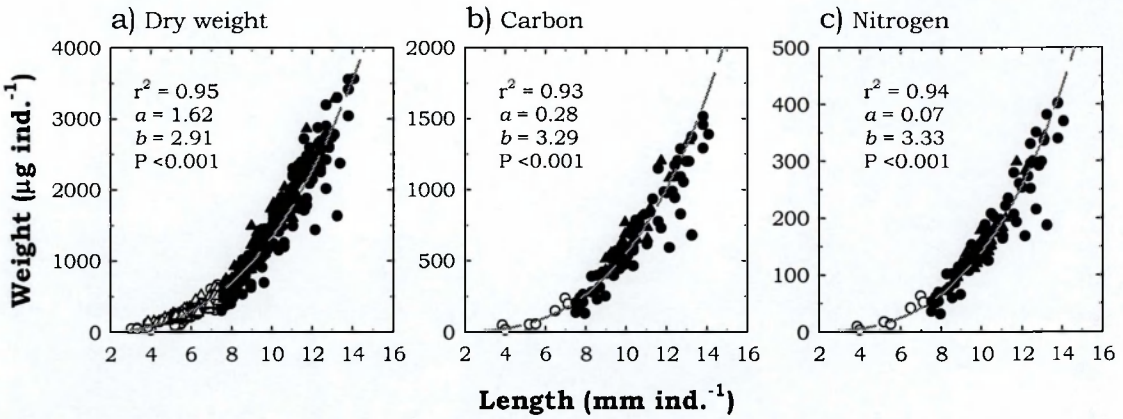

Figure 6.6 The relationship between the individual length of *Nyctiphanes australis* and the individual body weight, in terms of a) dry weight, b) carbon weight, and c) nitrogen weight. The lines of best fit ($Y = aX^b$) and their regression parameters are shown on each graph. Symbol colours define the developmental stage: sub-adults/adults = black, furciliae = white. Symbol shapes define the sampling date: Nov'00 = circles, Dec'00 = triangles.

little from their respective regression lines, implying that the samples collected during each sampling visit consisted of individuals with highly similar length/weight body conditions.

Figure 6.7 shows that the body weight (dry, carbon or nitrogen weight) of size-normalised (10 mm-long) *N. australis* did not increase consistently with increases in gut fullness. Simple (least squares) linear regression analysis of gut fullness (X_i values) versus body weight (Y_i values) showed that the slope of each line, b, did not differ significantly from zero at the $\alpha = 0.05$ level (b = -0.63 to 0.76; ANOVA: P = 0.19 to 0.98). This implies that the gut contents of *N. australis* were not significant in terms of the overall mass of the individual, and therefore that defaecation would not represent a significant avenue for carbon and nitrogen loss in this species.

Figure 6.8 shows that the carbon and nitrogen weight of *N. australis* increased linearly with increases in dry weight (i.e. $Y = a + bX$, $b > 0$). Furthermore, these positive relationships were strong ($r^2 > 0.98$) and highly significant (ANOVA: P <0.001),

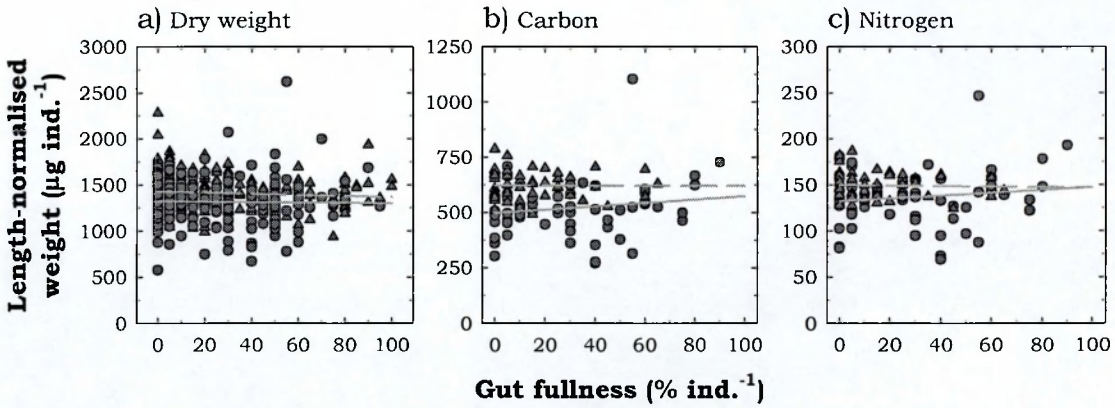

Figure 6.7 The relationship between the individual gut fullness of *Nyctiphanes australis* (furciliae, sub-adults and adults combined) and the length-normalised (10 mm long) individual body weight, in terms of a) dry weight, b) carbon weight, and c) nitrogen weight. The lines of best fit ($Y = a + bX$) are shown on each graph (bold lines = Nov'00, broken lines = Dec'00). See Figure 6.6 for an explanation of the symbol shapes.

suggesting that dry weight might represent a proxy for both carbon and nitrogen weight. Therefore, where dry weight but not carbon or nitrogen weight measurements were made on an individual (November: 148 out of 216 individuals; December: 188 out of 238 individuals), these relationships were applied to predict the carbon and nitrogen weight, and therefore increase the sample size for more robust statistical analysis.

Figure 6.9 shows that the nitrogen weight of *N. australis* increased linearly with increases in carbon weight (i.e. $Y = a + bX$, $b>0$). Moreover, the C:N (atoms) of all individuals was found to be highly uniform (mean±1SD = 4.60±0.34), implying that the carbon/nitrogen body condition of all individuals was very similar.

Variability in the data

Table 6.4 shows the mean, standard deviation (SD) and coefficient of variation (V) of the various biometric measurements made on *Nyctiphanes australis* collected in Deep Cove during the period November to December 2000. The data were combined from each sampling date, but considered separately for sub-adults/adults and furciliae. The

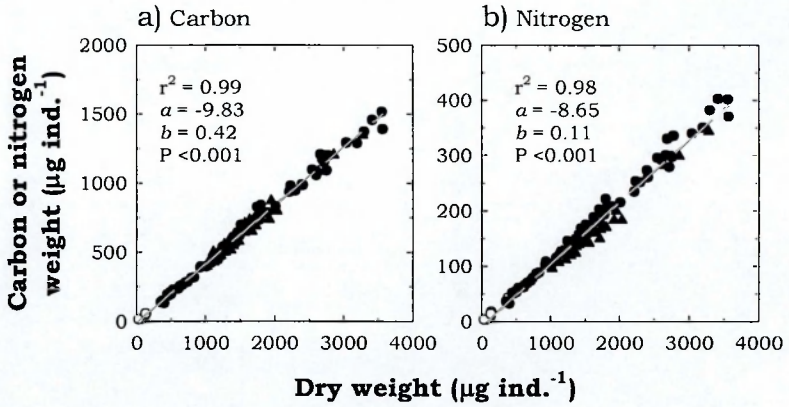

Figure 6.8 The relationship between the individual dry weight of *Nyctiphanes australis* and the individual elemental composition, in terms of a) carbon weight, and b) nitrogen weight. The lines of best fit ($Y = a + bX$) and their regression parameters are shown on each graph. See Figure 6.6 for an explanation of the symbols.

size-normalisation of the dry-, carbon- and nitrogen-weight measurements to a mean length (eye-telson) of 10 mm (see section 2.3.6) strongly reduced V in all but one instance, demonstrating that differences in the size of individuals between samples represent a significant source of variability in the data, and highlighting the importance of performing the size-normalisation procedure. Normalisation to a mean dry weight (1.5 mg) reduced the variability in carbon and nitrogen weight even more.

The size-structure of the N. australis population from body length measurements

Figure 6.10 shows the length distribution of *N. australis* collected in November and December 2000. The lengths of measured specimens ranged from 3.03 to 14.07 mm in November, and from 3.86 to 13.10 mm in December. From the descriptions of Sheard (1953), these length ranges encompassed furciliae (1.9-7.5 mm), sub-adults (7.5-11 mm) and adults (>11 mm) in both months. In November, there was a noticeable difference between the length distribution of samples obtained during the day and during the night (Figure 6.10a). As mentioned above, more individuals were caught in

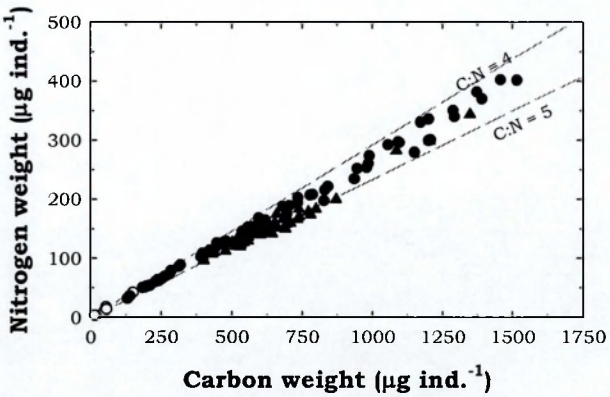

Figure 6.9 The relationship between the individual carbon weight of *Nyctiphanes australis* and the individual nitrogen weight. Lines show the C:N (atoms) lines of equivalence. See Figure 6.6 for an explanation of the symbols.

net tows made at night than during the day. Nighttime samples (n = 192) consisted predominantly of individuals >8 mm (i.e. sub-adults and adults), while daytime samples (n = 24) consisted predominantly of individuals <8 mm (i.e. furciliae and smaller sub-adults). The length data for December (Figure 6.10b) showed that, while catch numbers were again lower during the day, the length distributions of both day (n = 43) and night (n = 197) samples were similar. Nighttime samples in November were dominated by 11-12 mm individuals (i.e. smaller adults), while those in December consisted mainly of smaller, 5-10 mm individuals (i.e. furciliae and sub-adults).

Diel patterns of feeding from gut fullness measurements

Given that a mixture of furciliae, sub-adults and adults were collected and measured (Figure 6.10), and that these stages may have been behaving differently, it was thought better to treat the gut-fullness data separately for sub-adults/adults (i.e. individuals >7.5 mm long) and furciliae (i.e. individuals ≤7.5 mm long).

Figure 6.11 shows the gut-fullness distribution of sub-adults/adults collected from the field at different times of the day and night in November and December 2000. In

Parameter	Analysis	Mean±1SD (*V* %)	
		Sub-adults/adults	Furciliae
Length (mm)		9.81±1.60 (16.32)	5.83±0.83 (15.89)
Dry weight (µg)	Raw measure	1385.4±710.5 (51.3)	297.3±126.6 (42.6)
	L-norm.	1363.6±223.5 (16.4)	1428.5±633.5 (44.4)
Carbon weight (µg)	Raw measure	666.1±311.1 (46.7)	97.1±85.7 (88.3)
	L-norm.	564.1±98.4 (17.5)	580.4±263.5 (45.4)
	DW-norm.	625.1±34.3 (5.5)	627.2±7.9 (1.3)
Nitrogen weight (µg)	Raw measure	170.0±83.8 (49.3)	26.2±23.5 (90.0)
	L-norm.	142.5±21.6 (15.2)	154.4±61.0 (39.5)
	DW-norm.	159.1±12.5 (7.9)	167.4±2.8 (1.7)

Table 6.4 The variability of the biometric measurements made on *Nyctiphanes australis* collected in Deep Cove in November and December 2000. "L-norm." and "DW-norm." refer to those measurements that were standardised to a mean body length (10 mm) or dry weight (1.5 mg), respectively, using ANCOVA (see section 2.3.6).

November, there were significant differences between the median gut fullness values at each time-point (Kruskal-Wallis: $H_3 = 21.307$, $P < 0.001$; Table 6.5). During the hours of darkness (21:50-04:35 h), the gut fullness of individuals, which were caught mostly in the top 40 m, ranged from 0 to 95 %, indicating that at least some of the population had been feeding well. By first light (05:35 h, 0-110 m), the guts of most individuals were <10 % full, suggesting that levels of feeding had already decreased by this time.

During the day (15:45-19:00 h, 0-110 m) only three individuals were caught and measured: two had empty guts, while one was 60 % full, hinting that at least some individuals may have been feeding during the day. From last light (21:50 h) onwards, the gut fullness of individuals in the surface waters began to increase, indicating a return to nighttime feeding levels: at 21:50 h (0-110 m) the gut fullness range was 0-5 %, and by 23:20 h (0-30 m) this range had increased to 0-95 %.

In December, there were also significant differences between the median gut-fullness

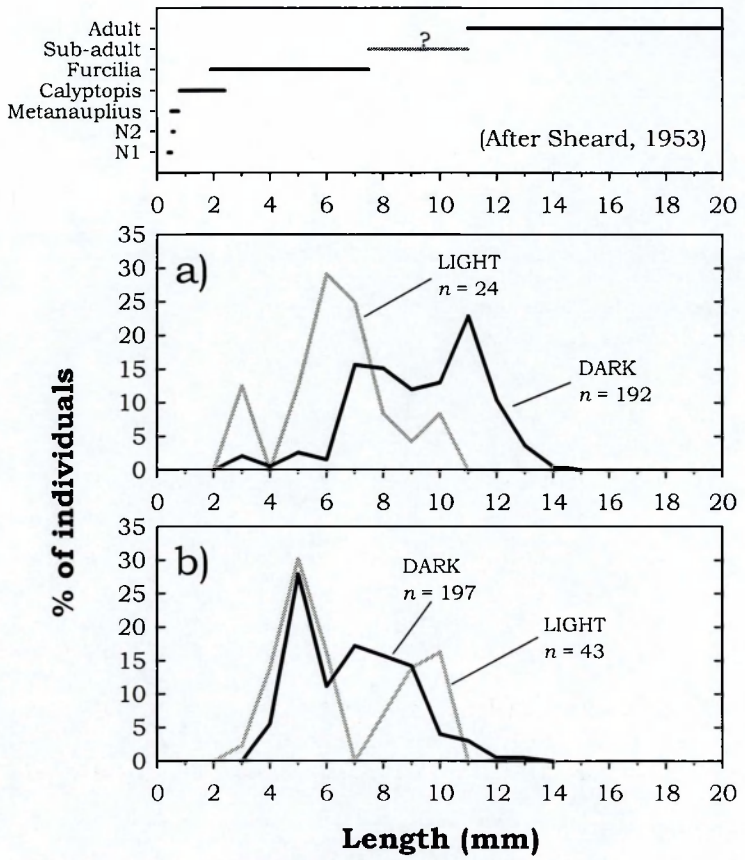

Figure 6.10 The length frequency (1 mm length classes, where 2 = 2 to <3 mm inclusive, and so on) of *Nyctiphanes australis* from Deep Cove in a) November 2000, and b) December 2000. Grey lines represent those individuals caught during daylight hours (between sunrise and sunset). Black lines represent those individuals caught during the night (between sunset and sunrise). The upper graph shows the length ranges of each developmental stage, after the measurements of Sheard (1953): ? represents data not explicitly included in this reference.

values at each time-point (Kruskal-Wallis: H_3 = 39.824, P <0.001; Table 6.5). As in November, individuals in the surface waters during the hours of darkness (00:55 h, 0-30 m) ranged from 0 to 80 % full, which was again indicative that at least a proportion of the population had been feeding well.

Individuals in the 'dawn' sample (05:00-05:10 h, 0-50 m) ranged from 0 to 60 % full, suggesting that some individuals were still feeding at the time of capture. During the day (15:20 h, 0-110 m), gut fullness did not exceed 5 %, suggesting that very little or no

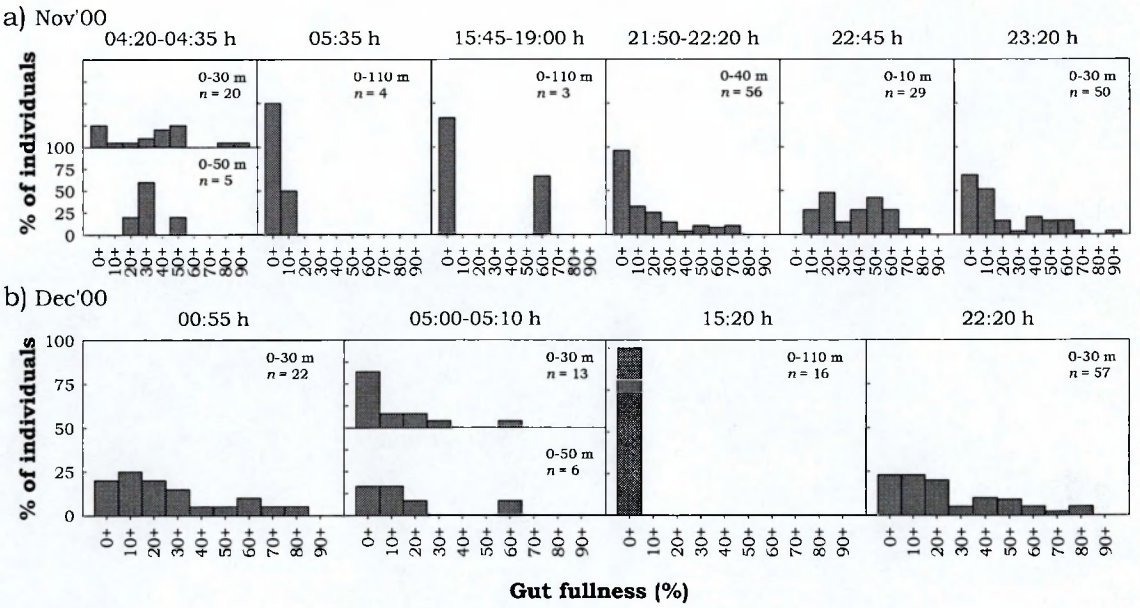

Figure 6.11 The gut-fullness distribution of *N. australis* (sub-adults/adults) collected at different times of the day and night in Deep Cove in a) November 2000, and b) December 2000.

feeding was occurring at this time. Individuals in the 'dusk' sample (22:20 h, 0-30 m) ranged from 0 to 85 % full, indicating that nighttime levels of feeding had already resumed by the time of capture.

Figure 6.12 shows the gut-fullness distribution of furciliae (≤7.5 mm) collected from the field at different times of the day and night in November and December 2000. In November, there were insufficient measurements to merit statistical analysis by Kruskal-Wallis, but there did appear to be a diel pattern in the median gut fullness values (Table 6.5). This pattern suggests that furciliae fed most actively during the hours of darkness. In December, where more samples were available for statistical analysis, there were significant differences between the median gut fullness values at each time-point (Kruskal-Wallis: $H_3 = 23.381$, $P < 0.001$; Table 6.5). Gut fullness was significantly lower in the day (Dunn's: $P < 0.05$) than at either dawn or dusk, suggesting, as in November, that furciliae fed most actively during the hours of darkness.

Date	Parameter	Dev. stage	Time-point			
			Night	Dawn	Day	Dusk
Nov'00	Time (h)		22:45-04:35	05:35-06:30	15:45-19:00	21:50-22:20
	Depth (m)		0-50	0-110	0-110	0-110
	Gut fullness median (%)	Sub-ad./ad.	25	0	0	5
		Furciliae				
	L-norm carbon mean (μg)	Sub-ad./ad.	540.00	442.78	444.13	539.70
		Furciliae	526.05	567.19	484.11	564.02
	L-norm nitrogen mean (μg)	Sub-ad./ad.	142.96	118.32	118.78	143.39
		Furciliae	133.70	152.54	132.35	151.35
Dec'00	Time (h)		00:55	05:00-05:10	15:20	22:20
	Depth (m)		0-30	0-50	0-110	0-30
	Gut fullness median (%)	Sub-ad./ad.	25	5	0	20
		Furciliae	25	35	5	40
	L-norm carbon mean (μg)	Sub-ad./ad.	578.19	557.50	626.03	606.78
		Furciliae	605.26	635.82	608.16	535.77
	L-norm nitrogen mean (μg)	Sub-ad./ad.	135.41	132.72	149.23	143.55
		Furciliae	121.27	123.26	110.00	109.39

Table 6.5 Diel changes in the median gut fullness and mean length-normalised ('L-norm' = 10 mm) carbon and nitrogen weight of *Nyctiphanes australis* (sub-adults/adults and furciliae) collected in Deep Cove in November and December 2000.

Diel changes in carbon and nitrogen weight

As with gut fullness, it was thought better to treat the carbon- and nitrogen-weight data separately for sub-adults/adults and furciliae. Figure 6.13 shows the carbon and nitrogen weight of size-normalised (10 mm-long) individual sub-adults/adults collected from the field at various times of the day and night in November and December 2000. In November, there were significant differences between both the carbon- and nitrogen-weight means at each time-point (carbon, ANOVA: $F_{3,174} = 4.954$, $P = 0.003$; nitrogen, ANOVA: $F_{3,174} = 4.662$, $P = 0.004$; Table 6.5). For both carbon and nitrogen, the most significant differences (Tukey's: $P < 0.05$) were found between the night and dawn means, and the dusk and dawn means. This pattern is consistent with the idea that individuals were feeding more at night than during the day. However, the fact that

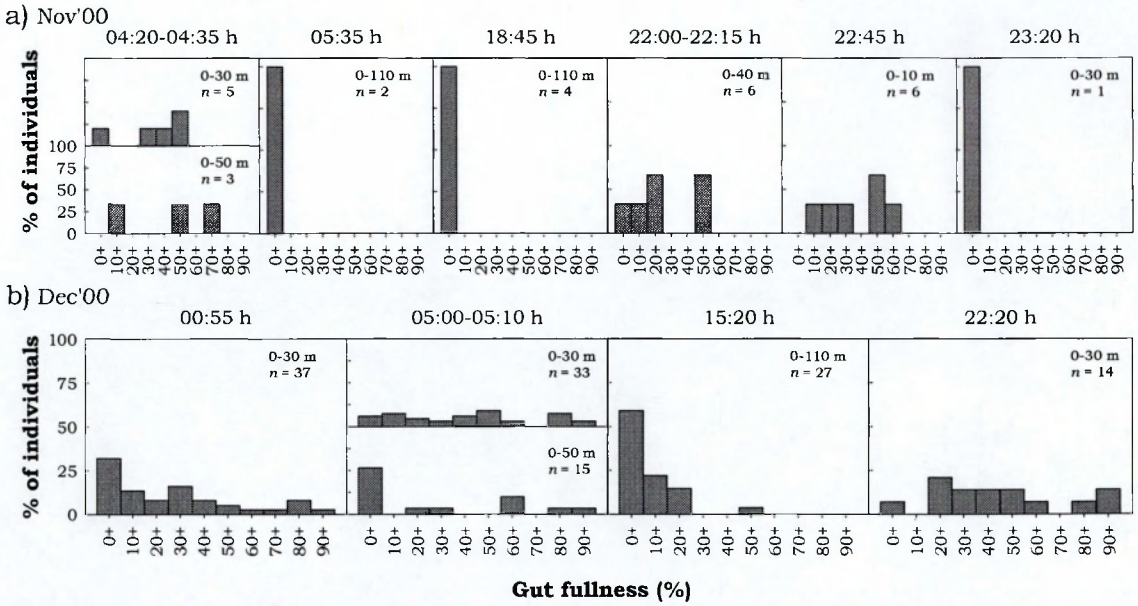

Figure 6.12 The gut-fullness distribution of *N. australis* (furciliae) collected at different times of the day and night in Deep Cove in a) November 2000, and b) December 2000.

individuals collected at dusk contained more carbon and nitrogen than those collected at dawn suggests that the dawn and dusk samples were not collected at the critical times/depths required for the application of ZOOFLUX. In December, there were also significant differences between the carbon- and nitrogen-weight means at each time-point (carbon, ANOVA: $F_{3,110} = 4.128$, $P = 0.008$; nitrogen, ANOVA: $F_{3,110} = 3.726$, $P = 0.014$; Table 6.5). For carbon, the most significant differences (Tukey's: $P < 0.05$) were found between the dawn mean (lower value) and those during the day and at dusk (higher values). For nitrogen, the most significant difference (Tukey's: $P < 0.05$) was found between the dawn mean (lower value) and that during the day (higher value). This pattern is not consistent with what one might expect from a pattern of nighttime feeding and daytime starvation.

Figure 6.14 shows the carbon and nitrogen weight of size-normalised (10 mm-long) furciliae collected from the field at various times of the day and night in November and December 2000. In November, there were no significant differences between either the

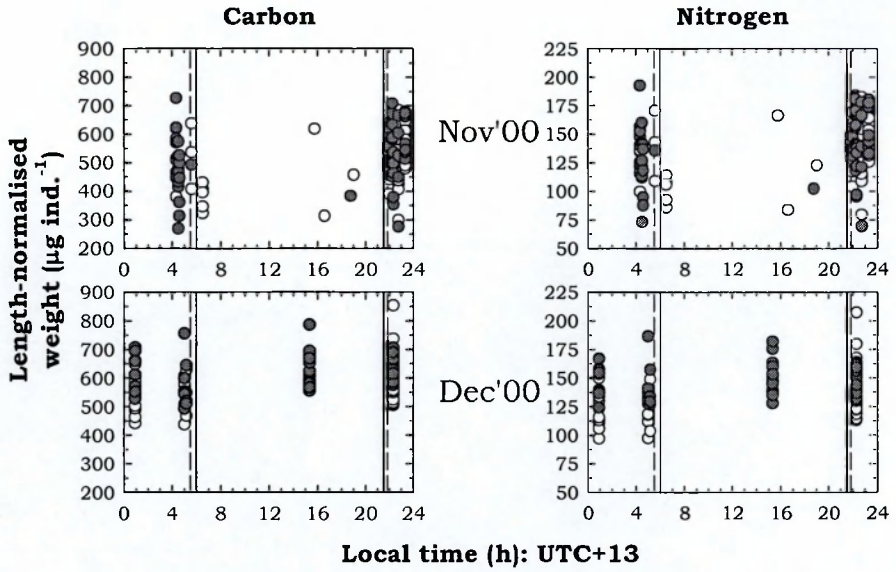

Figure 6.13 Diel differences in the carbon and nitrogen weight of size-normalised (10 mm long) *Nyctiphanes australis* (sub-adults/adults) collected in Deep Cove in November and December 2000. Broken lines represent the times of first and last light. Solid lines represent the times of sunrise and sunset. Filled symbols represent those values measured directly. Empty symbols represent those values derived from dry weight (see Figure 6.8 for the regression parameters used). Values represent n = 1 individual in both months.

carbon- or nitrogen-weight means at each time-point (carbon, ANOVA: $F_{3,33} = 0.409$, P = 0.748; nitrogen, ANOVA: $F_{3,33} = 0.421$, P = 0.739; Table 6.5), although the mean values do support the idea that furciliae fed most actively during the hours of darkness. In December, there were significant differences between the carbon- and nitrogen-weight means at each time-point (carbon, ANOVA: $F_{3,120} = 3.975$, P = 0.01; nitrogen, ANOVA: $F_{3,120} = 3.959$, P = 0.01; Table 6.5). For carbon, the most significant difference (Tukey's: P <0.05) was found between the dawn and the dusk means, while no significant differences were seen between the nitrogen means when compared directly with each other. However, this pattern of change is again consistent with what one might expect from higher levels of feeding during the night.

For both sub-adults/adults and furciliae, variability (V) in both carbon and nitrogen was high at each time-point. In November, V ranged from 15 to 30 % in sub-

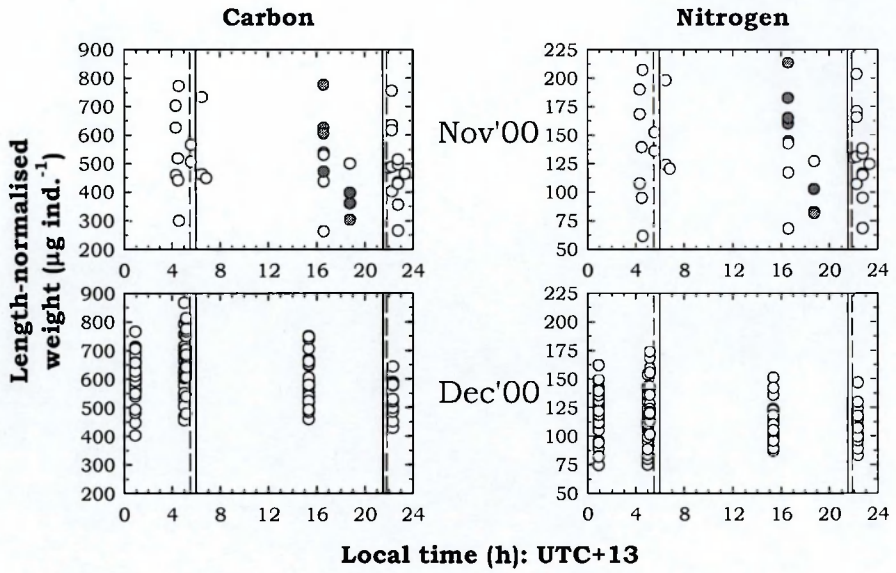

Figure 6.14 Diel differences in the carbon and nitrogen weight of size-normalised (10 mm long) *Nyctiphanes australis* (furciliae) collected in Deep Cove in November and December 2000. Broken lines represent the times of first and last light. Solid lines represent the times of sunrise and sunset. Filled symbols represent those values measured directly. Empty symbols represent those values derived from dry weight (see Figure 6.8 for the regression equations used). Values represent n = 1 individual in both months.

adults/adults, and from 20 to 40 % in furciliae. In December, V ranged from 10 to 15 % in sub-adults/adults, and from 10 to 20 % in furciliae. Within each time-point, this variability was enhanced by the inclusion of carbon and nitrogen values derived from dry weight measurements. For example, for sub-adults/adults collected at dusk in December, the V of the directly measured carbon values was 6 %, while that of the carbon values derived from dry weight was 11 %.

6.3.3 Statistical assessment of the success of ZOOFLUX in Deep Cove

Table 6.6 shows the statistical success of the third field-application of ZOOFLUX, based on *N. australis* (furciliae and sub-adults/adults) collected in Deep Cove in November and December 2000 (see section 2.3.8 for an explanation of the calculations used).

Date	Element	n	δ %	V %	ANOVA: P	β %	n_{min}	δ_{min} %
Sub-adults/adults								
Nov'00	Carbon	35	-22	18	0.001	n/a	14	14
	Nitrogen	35	-21	18	0.001	n/a	14	14
Dec'00	Carbon	38	-9	12	0.005	n/a	36	9
	Nitrogen	38	-8	13	0.022	n/a	54	10
Furciliae								
Nov'00	Carbon	5	<1	22	0.970	95	42500	52
	Nitrogen	5	<1	22	0.958	95	22300	52
Dec'00	Carbon	31	16	14	<0.001	n/a	19	12
	Nitrogen	31	11	17	0.032	n/a	48	14

Table 6.6 The statistical 'success' of ZOOFLUX in Deep Cove (see section 2.3.8), based on the carbon and nitrogen weight of size-normalised (10 mm-long) *Nyctiphanes australis* collected at dawn and dusk. n = the mean of the number of samples collected at dawn and at dusk. δ = the dawn-dusk difference in carbon or nitrogen weight. V = the mean coefficient of variation of the carbon or nitrogen data from both dawn and dusk. β = the probability of committing a Type II error (when ANOVA: P >0.05). n_{min} = the minimum number of samples required in order to detect a significant δ (ANOVA: P $\leq$0.05). δ_{min} = the minimum detectable dawn-dusk difference.

The number of samples available for the dawn-dusk comparisons (n, expressed as the mean of the number collected at both dawn and dusk) was relatively low (5 to 38 samples), while the variability in the carbon and nitrogen measurements (V, expressed as the mean of the coefficients of variation at both dawn and dusk) was relatively high (12 to 22 %). In four out of eight comparisons, the dawn-dusk difference (δ, expressed as a percentage change) was actually seen to be negative (i.e. carbon and/or nitrogen weight actually seemed to have increased during the day). In two out of eight comparisons, δ was found to be non-significant (ANOVA: P >0.05), and the probability that a Type II error had been made (β) was high in each case (95 %). The variability (V) in the data was also high in these instances (22 %), and sample numbers were low (5 samples).

Overall, the minimum number of samples that would have been required (n_{min},

expressed as the mean) in order to conclude that the observed dawn-dusk differences were significant (ANOVA: $P \leq 0.05$) ranged widely (14 to 42500 samples). In only four instances was this number less than the number of samples actually collected, although, with the exception of the furciliae data in November, these kinds of numbers (14 to 54 samples) are theoretically obtainable in the field. The magnitude of n_{min} related less to either the sample size (n) or the variability in the data (when expressed as V), and more to the particular source of the variability. Specifically, the higher the error MS in relation to the to the groups MS (see section 2.3.8), the higher the value of n_{min}, and *vice versa* (recall that Equation 2.11 employs only error MS as the variability term). That is to say, the variability within the data at either dawn or dusk was more important in deciding the minimum number of samples required than the variability between the data at each of these time-points. Finally, the minimum dawn-dusk difference that would have been detectable (δ_{min}) in each case ranged from 9 to 52 %.

6.4 Discussion

As with the previously reported field investigations carried out in both the Clyde Sea (Chapter 4) and the Sargasso Sea (Chapter 5), the fieldwork carried out in Doubtful Sound was primarily aimed at gaining a better understanding of the active flux of carbon and nitrogen in the marine environment. Like Inchmarnock Water, Deep Cove provided a convenient site for such an investigation (at least in terms of shelter, water depth and distance from shore), and again helped to address the idea that fiords might be usefully investigated as logistically-convenient proxies for more globally-typical open-ocean processes. The two previous zooplankton studies in Fiordland that have been reported in the literature (see section 6.1.4) have provided an idea of the type of mesozooplankton community that one might expect to find in Deep Cove. Of the two

most widely recognised vertical migrants found in these studies (i.e. *Nyctiphanes australis* and *Metridia lucens*), both were collected during the present study, although biometric measurements were only made on *N. australis* (since the presence of *M. lucens* was not recognised until well into the sampling programme). Data from depth-discrete net tows (Figure 6.5) revealed that the bulk of the mesozooplankton community was epipelagic, remaining in the top 30 m both night and day. It is unclear as to whether *N. australis* and/or *M. lucens* were performing DVM at this site. This issue is discussed further below. The biometric measurements of *N. australis* (Figures 6.6 to 6.9) revealed that this species of potential migrant, unlike those migrants collected in Inchmarnock Water (Figures 4.19 to 4.28) and at BATS (Figures 5.14 to 5.17), exhibited very little in the way of inter-individual physiological variability, at least during the relatively short study period. As with the previous approaches (sections 4.4 and 5.4), this information, along with measurements of the physical environment (Figures 6.3 and 6.4) and an assessment of at least some of the potential food sources available to *N. australis* (Table 6.1 shows potential metazoan prey, but *N. australis* is also a facultative herbivore), now allows a number of active-flux related questions to be posed: (1) Was the physical environment conducive to an active flux?; (2) How and why were the zooplankton behaving as they did, and what were the consequences of this to the ecosystem?; (3) Was an active export flux of carbon or nitrogen actually occurring at this site, and, if so, could it be quantified accurately using the present dataset? These questions are addressed in turn below.

6.4.1 The physical environment

It is immediately apparent that, whatever the nature and magnitude of material fluxes in this region, processes within Doubtful Sound will be of limited significance within

global-scale biogeochemical cycling due to the fiord's limited size (total surface area = 83.7 km^2). However, the purpose of this particular field visit, as in Inchmarnock Water, was to test the ZOOFLUX technique and to assess the suitability of fiord-based studies as proxies for open-ocean research.

Bathymetry

As in Inchmarnock Water, the water depth in Deep Cove, at ~125 m, represents a potential barrier to vertically migrating zooplankton. *N. australis* is capable of descending to depths of up to 400 m (e.g. Bartle, 1976), meaning that it may well have associated with the benthos during the day in Deep Cove. Indeed, McClatchie *et al.* (1990) discussed the possibility that this species associates with the benthos in Otago Harbour, given that its exoskeleton was found to be colonised by benthic diatoms. As discussed for krill in Inchmarnock Water (see section 4.4.1), there is a possibility that *N. australis* was feeding on resuspended bottom sediments during the day, given its known ability to consume detritus (e.g. Sheard, 1953), meaning that any active flux caused by nighttime feeding at the surface may well have been reduced, or even reversed, as a consequence of this behaviour. Certainly there were hints from the gut-fullness observations that at least some individuals may have been feeding during the day in November (Figure 6.11), although issues with net avoidance (see below) and the fact that the net tows did not sample the deepest 15 m of the water column mean that the existence of this potential benthic association could not be proved in this instance.

Hydrography

The likely absence of any pycnocline below the surface LSL in November and December (see Figure 6.3) would suggest that any material carried to depth would not

have been sequestered there for any length of time (based on the understanding that the pycnocline is the primary barrier to upward mixing). Moreover, previous observations of the structure of the water column over the course of the year (from CTD data, not shown here, for the period April 1999 to February 2000 kindly provided by P. Brewin) have shown that such a pycnocline is probably never established in Deep Cove. That said, it has been suggested that there may be some inhibition of deep-water flow at the head of Doubtful Sound, given the fact that the Deep Cove sill (55 m) is somewhat shallower than the main sill (101 m) at the mouth of the sound (Stanton & Pickard, 1981). The typical pattern of estuarine circulation may also act to concentrate material in the deeper waters of Deep Cove. These considerations might mean that at least a proportion of the deep-water material in Deep Cove will have time to sink or diffuse to the benthos, where it may become consolidated for longer periods of time (but recall the potential for benthic feeding by *N. australis* discussed above). However, second-order mixing and entrainment effects resulting from the velocity shear between the LSL and the deeper layer (Gibbs *et al.*, 2000) provide one way in which deep-water material might be removed into the seaward-flowing LSL, thereby causing the magnitude of any export flux to be reduced.

The dominant hydrographic feature in Deep Cove is the surface LSL, which contains high levels of dissolved and particulate material, primarily of terrigenous origin. In terms of the active flux, this feature may be important in two ways. Firstly, it is a potentially significant source of nutrients for the deeper waters. The remarkably steep density gradient at the base of the LSL means that particulates within the LSL are often unable to penetrate to depth via passive processes, often resulting in a noticeable layer of material a few metres below the surface. Indeed, Whitman and Grange (1998) also mentioned that the base of the LSL is visible to SCUBA divers "as a density and

coloration discontinuity". It is therefore possible that, despite containing high levels of terrigenous material, this feature is acting to 'insulate' the deeper waters from inputs of material from above. However, it appeared likely that *N. australis* was migrating into the 5-10 m depth interval at night, based on the observations from net tows made in November (see section 6.3.2). Given that the LSL extended to at least 9 m at this time (Figure 6.3), it is therefore possible that this potential migrant could have ingested some of the material within this layer and transported it to depth following descent. Secondly, the LSL is likely to have an effect on the sub-surface light regime which, in turn, is likely to influence the nature of zooplankton VM behaviour. This connection was briefly mentioned by Hays *et al.* (1998), who suggested that the typically high levels of humic material in the LSL (as described by Davies-Colley, 1992) might act to reduce the extent of DVM by reducing the light levels within the water column. No work has been carried out to test this hypothesis at this site. Indeed, it is evident that Deep Cove represents an interesting site for future studies on the influence of light on DVM.

Given the potential importance of the LSL to the flux of material within the Deep Cove ecosystem, it is important to understand the dynamics of this layer. As Gibbs *et al.* (2000) demonstrated, the LSL is affected by wind-stress and precipitation, and may show noticeable variability from one hour to the next. The somewhat confusing light profile in the top 9 m in November 2000 (Figure 6.4), whereby light levels actually appear to increase between 3 and 4 m, could be a reflection of the highly variable nature of the optical properties within the dynamic LSL. That is to say, light absorption might vary over short timescales (seconds to minutes) at a given point as patches of water containing more or less humic material pass by in the current. It is also possible, however, that the light meter was not sufficiently accurate. The marked differences in the profiles of temperature and salinity between November and December 2000 (Figure

6.3) also highlight the strong variability inherent in the LSL. The observation of slightly fresher water in the top 4 m in November is most likely due to the fact that the hydro-electric facility 'tailrace' was operative at this time, but had been switched off by the time sampling resumed in December. It is feasible that this would have had an impact on the mesozooplankton community, as was shown by Kaartvedt & Svendsen (1990, 1995) for a similar situation in a Norwegian fjord. The fact that there was still a marked surface LSL in December, despite the lack of freshwater input from the tailrace, demonstrates that natural runoff and precipitation are also significant in the maintenance of this feature in Deep Cove. The effect of the tailrace was also evident in the temperature profiles. In the absence of such a significant input of cooler water in December, surface temperatures were ~4 °C warmer than in November. By way of interest, Doubtful Sound is unique within Fiordland with respect to surface temperature. In the other fiords, the freshwater runoff is typically warmer than the underlying fiord water. In Doubtful Sound, the dominant freshwater input from Lakes Te Anau and Manapouri means that the surface waters in this case are actually cooler than those underneath. It is also possible that the differences in the magnitude of light absorption between months (Figure 6.4) were related to the switching-off of the tailrace. Absorption was lower when the tailrace was active, suggesting that less humic material is present in the lake water than in the natural run-off. As Davies-Colley (1992) discussed, the high levels of "yellow substance" in the LSL of Doubtful Sound are due to the "intense staining of freshwater drainage by humic substances leached out of the Fiordland soils". One might suggest that the soils to the east of Wilmot Pass, which will form the catchment for Lakes Te Anau and Manapouri, are less rich in these humic substances. Given that such a dominant feature of the ecosystem (i.e. the tailrace) can be manipulated, Deep Cove might be considered suitable as a natural mesocosm conducive

to a variety of controlled ecological experiments.

6.4.2 Mesozooplankton behaviour and ecosystem consequences

The same points raised for both Inchmarnock Water (section 4.4.2) and the BATS site (section 5.4.2) also apply to Deep Cove: if we can understand the way in which the zooplankton community was behaving and why, then we can understand better the processes controlling whether or not an active flux would have been occurring. The mesozooplankton community in Deep Cove was investigated through net tows (500 μm mesh). Acoustic instruments have also been moored in Doubtful Sound at various times (150 kHz Sontek ADCP). However, a precursory analysis of these data (kindly provided by M.T. Gibbs and H. Bowman) indicated that the mooring sites were probably not close enough to the present study site for these datasets to represent a useful line of further enquiry, at least in this instance. The net-catch data (Table 6.1) showed that the mesozooplankton community consisted of both neritic and open-water taxa, with a strong contribution by holoplanktonic crustacea (calanoid copepods in particular). Stratified day/night tows (Figure 6.5) suggested that most taxa were epipelagic, remaining in the top 30 m of the water column both day and night. However, these data also suggest that a number of taxa may have been performing DVM to varying extents, the most noticeable candidates being *Clausocalanus* spp., barnacle larvae and mysids. As for the known migrant species *N. australis* and *M. lucens*, the picture is unclear for a number of reasons, which we shall now turn to.

Interpreting vertical migration behaviour from the net-catch data

As discussed in sections 4.4.2 and 5.4.2, the relatively low spatial and temporal resolution of the net tows limited the ability to describe the precise amplitude and

timing of DVM in Deep Cove. However, in the case of many of the taxa identified, the daytime and nighttime vertical distributions did not appear to be markedly different (Figure 6.5): the highest concentrations were found in the top 30 m both day and night, suggesting an epipelagic lifestyle. For those taxa that did show different day/night patterns in their vertical distribution, one can be less sure of their behaviour. It is likely that these differences were due to DVM behaviour, but the possibility of sampling artifacts such as net avoidance and patchiness cannot be ruled out. For *N. australis*, which was measured further for body length, gut fullness and dry, carbon and nitrogen weight, there is also the possibility of inferring the vertical movements of this species using the "tracer" techniques highlighted by Pearre (1979a, 2003) and discussed in section 4.4.2. As in the Clyde Sea study, however, stratified data of this nature were limited, such that these tracers were again of limited use in this instance.

In the case of *N. australis, M. lucens* and mysids (and also decapod larvae in November only), far fewer individuals were caught during the day, particularly in November (Table 6.1). This cast doubt on the quantitative accuracy of daytime sampling for these taxa. For *N. australis*, mysids and decapod larvae, at least, one might suggest that their enhanced visual acuity and faster swimming speeds would have allowed them to avoid the white, 1 m WP-2 net to a certain degree. Hosie & Ritz (1983) discussed similar issues when sampling for *N. australis* in Storm Bay, Tasmania. Certainly the fact that predominantly smaller (<8 mm) *N. australis* were caught by day in November (Figure 6.10) would suggest that larger, faster-swimming individuals (sub-adults and adults) were able to avoid the net during daylight hours. This suggestion is supported by the observation that equally large individuals were caught both day and night in December (Figure 6.10), when levels of light absorption were higher (Figure 6.4) and when one might therefore have expected the net to have been less noticeable.

The fact that the day/night differences in the catch numbers of all four taxa were much more pronounced in November (Table 6.1), when the water was clearer, provides further confirmation of this theory. For *N. australis*, it is unclear, however, as to whether the catching of mainly smaller individuals by night in December (5-10 mm, i.e. furciliae and sub-adults) than in November (11-12 mm, i.e. adults) (Figure 6.10) reflected a true change in the modal size of the population, or whether a degree of net avoidance was also occurring at night in December. The increased presence of calyptopes and furciliae in December (Table 6.1) might suggest the former explanation.

There are a variety of other possible reasons as to why daytime catches were so poor. It may have been that daytime distributions were much more diffuse than at night, or that individuals moved away from the study site by day and returned at night. Given that net tows were not made deeper than 110 m (to avoid catching the net on the seafloor), it is also possible that the undersampling of these taxa may have been due to their being found deeper than 110 m during the day. This is more likely to have been the case for *N. australis*, *M. lucens* and mysids, and less likely for decapod larvae, given that the former taxa were more likely to have been performing DVM (based on numerous observations in the literature), while the latter in fact appeared to be more epipelagic (see Figure 6.5). Indeed, Ritz & Hosie (1982) discussed the potential for inefficient sampling of *N. australis* adults at various times of the year in Storm Bay, Tasmania, due to association with the benthos. The possibility that *N. australis* will have been associating with the benthos during the day in Deep Cove, and the consequences of this behaviour in terms of the active flux, were discussed above (section 6.4.1).

Mesozooplankton DVM behaviour and the potential for an active flux to occur

The bulk of the zooplankton community in Deep Cove during the present study

inhabited the upper 30 m of the water column (Figure 6.5), and would therefore not have been important within considerations of an active flux. However, given the indications that a number of taxa may well have been performing DVM to various degrees, it is possible that each of these may have contributed in part to an active transport of material to deeper water. The following discussion relates to the possible DVM behaviour of *N. australis*, since this was the species chosen for the present application of ZOOFLUX.

The vertical migration strategy of N. australis

It is apparent that the data gathered here can only provide a tentative assessment as to whether or not *N. australis* was performing DVM in Deep Cove. Nighttime net tows to 110 m revealed that the bulk of the population (97 % in November, 55 % in December) was to be found in the top 30 m during the hours of darkness. Furthermore, as discussed in section 6.3.2, much of the population may well have concentrated in the 5-10 m depth stratum, at least in November. This is consistent with the behaviour one might expect from an omnivorous diel migrant that ascends to feed on phytoplankton and epizooplankton in the euphotic zone under cover of darkness. This is also supported by the gut-fullness observations (Figures 6.11 and 6.12), which suggested higher levels of feeding at night in both furciliae and sub-adults/adults.

However, the fact that ~45 % of the nighttime population in December was to be found deeper than 30 m also suggests that this species, in common with many other diel migrants, exhibits a degree of individual variability in its vertical distribution at any one time. While the striking uniformity in the length/weight and carbon/nitrogen relationships of individuals at this time (see Figures 6.6 and 6.9) might suggest that this distributional variability was not driven by differences in body condition, it must be

noted that these samples were only obtained from the top 50 m. It would certainly be interesting to sample individuals from the deeper-water fraction of the population as an attempt to understand the causes of this variability (see Hays *et al.*, 2001a, for an example of a similar study on deep nighttime residence in a marine copepod).

As for knowing the daytime distribution, the discussion above highlighted the potential uncertainties associated with both daytime net avoidance and/or possible undersampling of a proportion of the population that may have been associating with the benthos during the day. Certainly, *N. australis* appeared to be performing DVM at the population level. However, the fact that at least some specimens were caught in the top 70 m during the day (Figure 6.5), and that a significant proportion of these were to be found in the top 30 m, indicates (as Hays *et al.*, 1998, found for *M. lucens* at this site) that at least some individuals within the population did not descend to the assumed safety of the depths. Hays *et al.* (1998) ascribed this behaviour in *M. lucens* to individuals with a lower body condition (carbon/length or nitrogen/length) needing to risk visual predation in order to build up sufficient energy reserves for successful reproduction. The same causation may also be applying to *N. australis*. However, it may also be the case that, due to the unusually high light absorption in Deep Cove, visual predation risk at this site is minimal. If this were the case, one would need to find alternative explanations as to why DVM was being undertaken.

6.4.3 Was an active flux occurring, and could it be measured with the current dataset?

The present study in Deep Cove has revealed novel information regarding the marine ecosystem here. Indeed, this appears to be the first study to have been conducted on the composition and vertical distribution of the mesozooplankton community, thereby usefully adding to the knowledge-base that exists for other aspects of the biological

regime in this fiord (see section 6.1.2 for references). Building on the ecological theme initiated by Hays *et al.* (1998), the considerations provided here have demonstrated the potential for future studies in this unique fiord to address a number of interesting topical issues concerning the ecology of marine zooplankton (and indeed other aspects of the environment). The unusually strong light-absorbing qualities of the water column, which allows deeper-water benthic species such as *Antipathes fiordensis* and *Astrobrachion constrictum* to exist here at atypically-shallow depths, is also likely to affect the behaviour of the pelagic community, beginning with the phytoplankton and ending with commercially-important fish and their predators, such as penguins and dolphins. Indeed, Doubtful Sound could potentially be used as a wonderful natural mesocosm: the environment shares a number of open-ocean-like properties, such as deep water and the presence of mesozooplankton with open-water affinities, but at the same time is restricted in size and can potentially be manipulated by controlling the input of freshwater from the hydro-electric tailrace. As the present study has revealed, altering the tailrace input represents one way of altering the light regime, a useful parameter to be able to control given its importance in the behaviour of most marine organisms. That said, it is apparent that this is a particularly sensitive habitat, and care should be taken to employ non-invasive study techniques where at all possible. Based on the information gathered thus far, the main focus of this study can now be addressed: would an active flux have been occurring in Deep Cove, and could this potential flux have been successfully measured with the present dataset?

Evidence for flux-conducive behaviour

The various considerations outlined above have shown that we can neither prove nor refute the existence of an active flux in Deep Cove. Certainly the lack of a pycnocline to

act as a barrier to mixing would suggest that deep-water material will not be sequestered for any length of time, and there are a wealth of unanswered questions that still remain regarding DVM at this site. Regarding *N. australis* in particular, the lack of a significant relationship between the gut fullness and weight of individuals (Figure 6.7) would suggest that the active defaecatory flux of POC and PON would be relatively unimportant in this species, while previous studies by Lasker (1964), Ritz & Hosie (1982) and Hosie & Ritz (1983) would suggest that the active moulting flux of POC and PON would be of greater relative importance. However, the assumed potential for benthic feeding by this known detritivore also means that any export flux caused by life-processes at depth may well be reduced, or even reversed. Recalling a similar suggestion for krill in the Clyde Sea, this might indicate that, despite their often synchronous and marked DVM behaviour, krill are not necessarily strong contributors to the active flux in fiords or, indeed, at any site where they can reach the seafloor.

Quantifying the active flux according to ZOOFLUX

The gut-fullness observations of both the furciliae and sub-adult/adults of *N. australis* in Deep Cove (Figures 6.11 and 6.12) supported the idea that a standard pattern of DVM might have been occurring, with more feeding occurring in the surface waters at night than in the depths during the day in both November and December. Moreover, the carbon- and nitrogen-weight measurements also supported this pattern for sub-adults/adults in November (Figure 6.13) and furciliae in both November and December (Figure 6.14), although it is unclear as to why sub-adults/adults actually contained more carbon and nitrogen during the day in December. However, only the furciliae showed the expected dawn-dusk decreases in carbon and nitrogen that are theoretically required for the quantification of the active flux, and these decreases were only significant

(ANOVA: $P \leq 0.05$) in December. As for the sub-adults/adults, both carbon and nitrogen actually showed dawn-dusk increases. As discussed above, it has not been possible to either prove or refute the idea that *N. australis* is undertaking DVM in Deep Cove. Therefore, the inability to describe significant (ANOVA: $P \leq 0.05$) dawn-dusk decreases in the carbon and nitrogen weight of this species in three out of four cases may simply be due to the fact that a classic pattern of DVM is not being performed. However, if DVM were being performed, then the most likely explanation is that migrants were not pinpointed at the exact times and depths at which they switched from higher feeding near the surface to lower feeding at depth. This was also discussed for both *Calanus* in the Clyde Sea (section 4.4.3), and *P. xiphias* and krill in the Sargasso Sea (section 4.4.3), and perhaps represents the most difficult practical consideration to overcome when undertaking field-sampling for ZOOFLUX. A more detailed understanding of the migrant community therefore appears to be necessary before specimen collections for ZOOFLUX can be carried out. However, as also found in both the Clyde Sea and Sargasso Sea, high variability and low sample numbers were again responsible for the statistical difficulties encountered at the data analysis stage (Table 6.5). This supports the suggestion made in section 5.4.3 that these potentially uncontrollable factors are common obstacles to the application of ZOOFLUX in the field. The issues surrounding the use of dry weight as a proxy for carbon and nitrogen weight are again the same as those discussed in sections 4.4.3 and 5.4.3.

In conclusion, *N. australis* had the potential to generate an active export flux of carbon and nitrogen in Deep Cove, based on diel changes observed in the gut-fullness and carbon and nitrogen weight of individuals, although any benthic feeding would act to reduce this flux. However, no pycnocline exists at this site below which deep-water material might be effectively sequestered, and uncertainties surrounding the migratory

behaviour of this species mean that the ability to quantify this potential active export according to ZOOFLUX was diminished in this instance.

6.4.4 Summary: the active flux in Deep Cove

1. *N. australis* performed NDVM at the population level, but some individuals remained at depth during the night, and others at the surface during the day.
2. For *N. australis*, defaecation did not represent a significant avenue for carbon and nitrogen loss relative to the mass of an individual.
3. The body condition (length:weight) of all *N.* australis collected during the present study was found to be uniform.
4. *N. australis* collected in the field at different times during the diel cycle showed evidence for a diel feeding rhythm, feeding at higher levels during the hours of darkness, and lower levels or not at all during the day.
5. For *N. australis* collected in the field at different times during the diel cycle, δ was found to be variable and V relatively high, while n was relatively low. The ability to measure the active flux at this site using ZOOFLUX would be improved with a better knowledge of the behavioural ecology of the migrating community.

7

A SYNTHESIS OF ACTIVE FLUX RESEARCH: THE PAST, PRESENT, AND FUTURE

7.1 Introduction

Two questions are posed in this synthesis chapter: (1) What have the present study and previous investigations revealed about the active flux?; (2) Where do we need to go from here?

In terms of what we know of the processes driving the active flux, it would be useful to summarise what we can now say about the relative importance of the various factors involved. This is addressed in section 7.2, in which a sensitivity analysis is presented based on data available for the open-ocean migrant *Pleuromamma xiphias*. As for how best to quantify the active flux, a critical evaluation of the ZOOFLUX technique employed during the present study is presented in section 7.3. This technique had initially promised to circumvent a number of issues raised during previous attempts to quantify the active flux, and this evaluation addresses the reasons as to why the first comprehensive field-applications of ZOOFLUX were not as successful as first hoped. Turning to the literature, a synopsis is presented in section 7.4 of those studies that have provided us with quantitative estimates of the active flux. This section concludes with suggestions as to the way in which field and laboratory efforts might be usefully and effectively directed in the future.

7.2 Factors influencing the active flux: a sensitivity analysis

Hays *et al.* (1997b) suggested that the active flux (of nitrogen in this case) "is probably mediated largely through nitrogenous excretion...rather than through defaecation". They also suggested that the rate of this flux will be influenced by other factors, such as inter-specific differences in excretion rates, regional and seasonal differences in water temperature, the length of time spent below the pycnocline each day and the size of the individual. One way to quantify the relative importance of the various factors involved

in the active flux is to conduct a model sensitivity analysis. Such an analysis is presented here, using a combination of data from the present and previous studies. The species used for this exercise was *Pleuromamma xiphias*, since it performs strong NDVM, and has been well studied at BATS. However, in theory, this exercise can be carried out for any vertical migrant of interest.

7.2.1 Model structure and parameters

This model assumes that vertical migrants will be found above the pycnocline at night and below this boundary by day. Carbon and nitrogen loading (% body C or N h^{-1}) will occur if individuals are feeding, while carbon and nitrogen loss (% body C or N h^{-1}) will occur via the defaecation of POC and PON, the excretion of DOC and TDN (total dissolved nitrogen = DON + NH_4^+), and the respiration of DIC. Defaecation rate is assumed to relate linearly to the rate of ingestion. Excretion and respiration rates are assumed to be weight-specific and linked to the mean environmental temperature, which, in August and September 2000 (BATS143 and BATS144, respectively), was 23 °C above the pycnocline and 18 °C below (Figure 5.8). A single 'run' of the model starts at dusk on day 1 and finishes at dusk on day 2. The initial carbon or nitrogen value used to 'kick-start' the model therefore represents the weight of an empty individual at dusk on day 1, while the dawn-dusk difference and the active flux are calculated from the net loss or gain of carbon or nitrogen between dawn and dusk on day 2.

The parameters adjusted included the initial carbon and nitrogen weight, the levels of feeding (night and day), the time spent at depth, and the migrating biomass. The effect of each of these changes in turn was monitored in terms of changes to the dawn-dusk difference, the active flux and the level of growth (in this context, 'growth' refers to the

difference between the carbon or nitrogen weight of an individual from dawn to dawn or dusk to dusk).

The carbon and nitrogen weight of vertical migrants

The carbon and nitrogen weight of *P. xiphias* adult males and females at BATS was recorded during the present study. This was either measured directly or extrapolated from dry weight measurements (Figure 5.23). Individual variability was found to be high, quite possibly reflecting differences in feeding history, with values ranging from 64 to 263 μg C ind.$^{-1}$, and from 15 to 73 μg N ind.$^{-1}$. Potential losses of both carbon and nitrogen during sample processing meant that these measurements might have underestimated the true body content by as much as 25 % (see section 5.3.4).

Ingestion rate

Schnetzer & Steinberg (2002a) estimated ingestion rates for *P. xiphias* at BATS based on gut fluorescence data (a measure of herbivory) and the carbon content of faecal material (a measure of carnivory). They found that 29 % of the POC defaecated was made up of plant material and that total ingestion rates ranged from 0.56 to 1.80 μg C ind.$^{-1}$ h^{-1}. With a carbon weight ranging from 64 to 263 μg C ind.$^{-1}$ (present study), this equates to an ingestion rate ranging from 0.21 to 2.81 % body C h^{-1}. In the absence of nitrogen-ingestion rates, similar values will be assumed for nitrogen.

Defaecation rate

Gut passage time (GPT) was estimated from two starvation experiments conducted at BATS during the present study, and found to range from 150 to 510 min (Figure 5.19). This agrees fairly well with a more sophisticated study at BATS, in which GPT was

found to range from 99 to 265 min (Schnetzer & Steinberg, 2002a). Both of these estimates, however, disagree strongly with the GPT of 25 to 30 min found by Arashkevich (1977). If we consider the complete range of GPT values (i.e. 25 to 510 min), and assume that two pellets occur simultaneously in the gut (Mauchline, 1998), we arrive at a defaecation rate that ranges from 0.3 to 4.8 pellets h^{-1}. The proportion of an individual's carbon and nitrogen weight defaecated from a single full gut is likely to be low (<1 %), based on the findings shown in Figure 5.15, and given that most of the gut carbon and nitrogen will be assimilated (after Carlotti & Hirche, 1997): in the present model, a single faecal pellet will be assumed to contain 0.2 % of the total body carbon or nitrogen of a copepod. A defaecation rate of 0.3 to 4.8 pellets h^{-1} will therefore equate to a loss of 0.06 to 0.96 % body C or N h^{-1}.

The number of faecal pellets produced by a copepod per unit time increases linearly with increases in ingestion rate (Mauchline, 1998). Therefore, at low levels of ingestion (0.21 % body C or N h^{-1}) one might expect to find low levels of defaecation (0.06 % body C or N h^{-1}): in this instance, ~2 % of ingested material is defaecated. Similarly, at high levels of ingestion (2.81 % body C or N h^{-1}) one might expect to find high levels of defaecation (0.96 % body C or N h^{-1}): in this instance, ~30 % of ingested material is defaecated. Based on these considerations, a mean defaecation rate of 10 % of the ingestion rate will be assumed in this model, i.e. an assimilation efficiency of 90 %.

Excretion and respiration rates

Adjusting for the effects of temperature

Literature values of metabolic rates are typically accompanied by the temperature at which they were measured. It is well known that temperature has "striking effects on many physiological processes" (Schmidt-Nielsen, 1983). Therefore, where the

experimental temperature (T_x) is specified for a published rate value (R_x), a corrected rate (R_y) must be calculated at the appropriate environmental temperature (T_y). If R_x is given at a single temperature (T_x), the calculation of R_y at T_y is made by assuming an appropriate Q_{10} value* and applying the equation:

$$R_y = R_x \times Q_{10}^{\frac{T_y - T_x}{10}}$$

Equation 7.1

However, if R_x is given at two different temperatures (i.e. R_{x1} at T_{x1}; R_{x2} at T_{x2}), R_y at T_y is still calculated from Equation 7.1, but this time using an actual Q_{10} value calculated from the equation:

$$Q_{10} = \left(\frac{R_{x2}}{R_{x1}}\right)^{\frac{10}{T_{x2} - T_{x1}}}$$

Equation 7.2

Equation 7.2 is only valid, however, if the two experimental temperatures are far enough apart to give reliable information about the temperature effect. In this model, it was decided that if the experimental temperatures were <5 °C apart, an appropriate Q_{10} was applied to both R_{x1} and R_{x2}, and the mean of the R_y values at T_{y1} and T_{y2} used. In all cases where an appropriate Q_{10} value was needed, a value of 2.5 was used (after Steinberg *et al.*, 2000).

* Q_{10} = the increase in a rate caused by a 10 °C increase in temperature

DOC and TDN excretion

P. xiphias at BATS has been found to excrete DOC at a mean rate of 0.9 μg C mg DW^{-1} h^{-1} at 26 °C (Steinberg *et al.*, 2000). Assuming a mean C:DW of 0.32, as measured during the present study (Figure 5.16a), this would equate to an excretion rate of 0.21 % body C h^{-1} within the top 160 m at night (23 °C), and 0.14 % body C h^{-1} below 160 m during the day (18 °C). Steinberg *et al.* (2002) also found that *P. xiphias* at BATS excreted TDN (= DON + NH_4^+) at mean rates of 0.43 and 0.54 μg N mg DW^{-1} h^{-1} at 21 and 26 °C, respectively. Assuming a mean N:DW of 0.09, as measured during the present study (Figure 5.16b), this would equate to an excretion rate of 0.52 % body N h^{-1} within the top 160 m at night (23 °C), and 0.42 % body N h^{-1} below 160 m during the day (18 °C).

DIC respiration

P. xiphias at BATS has been found to respire DIC at mean rates of 0.9 and 2.4 μg C mg DW^{-1} h^{-1} at 22 and 26 °C, respectively (Steinberg *et al.*, 2000). Assuming a mean C:DW of 0.32 (present study), this would equate to a respiration rate of 0.44 % body C h^{-1} within the top 160 m at night (23 °C), and 0.28 % body C h^{-1} below 160 m during the day (18 °C).

Time spent below the pycnocline each day by vertical migrants

The DVM movements of *P. xiphias* adults at BATS during the present study appeared to be cued by the changes in light levels occurring around dawn and dusk (Figure 5.12). The time spent below the mixed layer by this species is therefore likely to correlate closely with day length. For the period August to September at BATS, deep (>160 m) daytime residence by *P. xiphias* will therefore range from 13 to 16 h d^{-1}.

Migrating biomass

The number of individuals migrating out of the surface mixed layer around dusk in August and September was estimated using a combination of the BATS zooplankton time-series data (Figure 5.11), estimates of the percentage of each size fraction made up by *P. xiphias* (Table 5.3), and individual dry weight measurements made during the present study (see e.g. Figure 5.14a). These calculations revealed a migrating biomass of 42 ind. m^{-2} d^{-1} in August, and 12 ind. m^{-2} d^{-1} in September (see section 5.3.4).

Other parameters

The passive sinking flux values used for comparison were the means of the fluxes measured at 200 m as part of the BATS programme in August and September 2000 (BATS143 and BATS144, respectively): POC = 14.94 mg C m^{-2} d^{-1}, PON = 1.73 mg N m^{-2} d^{-1} (Figure 5.10). Similarly, the mean depth-integrated (0-140 m) primary production values from these two cruises was also used: ^{14}C uptake = 453.34 mg C m^{-2} d^{-1} (Figure 5.9). The surface area of the Sargasso Sea used for extrapolation of the total active flux was 5.12×10^{12} m^2.

7.2.2 Model results

Table 7.1 shows the results of seven separate 'runs' of the model for both carbon and nitrogen, varying one parameter at a time and monitoring its effect on the various aspects of the active flux. Temperature was maintained at 23 °C above the pycnocline and 18 °C below.

CN	Ingestion		DRT	MB	δ	AF	AF/PF	AF/PP	G	TF
μg	% body C or N h^{-1}		h	ind. m^{-2}	%	μg m^{-2} d^{-1}	%	%	% d^{-1}	Pg C or N y^{-1}
	Night	Day								
Carbon										
64	1.3	0	13	12	5.4	43.6	0.3	0.01	0	0.00008
64	1.3	0	16	12	6.6 *	52.8 *	0.4 *	0.01 *	-2.8 *	0.00010 *
64	1.3	0	16	42	6.6	184.9 *	1.2 *	0.04 *	-2.8	0.00035 *
263	1.3	0	16	42	6.6	759.6 *	5.1 *	0.17 *	-2.8	0.00142 *
263	2.6	0	16	42	6.6	827.8 *	5.5 *	0.18 *	6.0 *	0.00155 *
263	2.6	0.65	16	42	-2.7 *	-343.6 *	n/a	n/a	16.6 *	-0.00064 *
263	2.6	0.65	13	42	-2.2 *	-291.6 *	n/a	n/a	21.2 *	-0.00054 *
Nitrogen										
15	1.2	0	13	12	5.4	10.3	0.6	n/a	0	0.00002
15	1.2	0	16	12	6.7 *	12.5 *	0.7 *	n/a	-2.8 *	0.00002 *
15	1.2	0	16	42	6.7	43.7 *	2.5 *	n/a	-2.8	0.00008 *
73	1.2	0	16	42	6.7	212.6 *	12.3 *	n/a	-2.8	0.00040 *
73	2.3	0	16	42	6.7	229.7 *	13.3 *	n/a	5.0 *	0.00043 *
73	2.3	0.6	16	42	-1.7 *	-58.4 *	n/a	n/a	14.4 *	-0.00011 *
73	2.3	0.6	13	42	-1.4 *	-49.5 *	n/a	n/a	18.8 *	-0.00009 *

Table 7.1 The results of a model to calculate the contribution of *P. xiphias* adults to the active flux of carbon and nitrogen in the Sargasso Sea, based on data from the BATS site in August/September 2000. Underlined values show where a parameter has been changed from that in the entry directly above. Asterisks (*) show where a result was altered by this change from the one directly above. Negative values indicate an upwards flux of material. CN = the initial carbon or nitrogen weight of an individual. DRT = deep residence time, i.e. the amount of time spent below the pycnocline each day. MB = migrating biomass. δ = the dawn-dusk difference in carbon or nitrogen weight. AF = active flux. AF/PF = active flux/passive flux. AF/PP = active flux/primary production. G = growth. TF = the total flux over the whole of the Sargasso Sea.

Minimum dietary requirements of carbon and nitrogen

For an individual that did not feed during the day, the minimum nighttime ingestion rates (for a 13 h night) required to balance the daily metabolic losses (i.e. to give a growth value of 0 %) were 1.3 % body C h^{-1} and 1.16 % body N h^{-1}. Note that these rates assume an assimilation efficiency of 90 %: efficiencies lower than this would therefore require higher minimum ingestion rates, and *vice versa*. Comparing these values to the range of ingestion rates suggested above (i.e. 0.21 to 2.81 % body C or N

h^{-1}), it is apparent that these threshold rates represent a relatively high level of feeding (40-45 % of the maximum rate). It follows that any feeding at depth would reduce the minimum amount of feeding required at night.

The dawn-dusk difference in carbon or nitrogen weight

The percentage change in an individual's carbon or nitrogen weight during the day while at depth depended on the amount of time spent there, and whether or not it was able to feed during this time. This is to be expected, since the longer an individual spends at depth, the more material will be released there through the continuing processes of excretion and respiration. In this case, a 3 h increase in the time spent at depth increased the dawn-dusk difference in carbon weight from 5.4 to 6.6 %. Any feeding at depth will act to reduce the net metabolic loss of carbon and nitrogen, and therefore the magnitude of the dawn-dusk difference. In this model, the effects of daytime feeding were striking: at only 25 % of the nighttime rate of feeding, the dawn-dusk difference in carbon weight changed from 6.6 % (no feeding) to –2.7 % (feeding). That is to say, levels of feeding at depth do not have to be particularly high before the export flux is reversed and an upward flux actually occurs. This echoes the point made by Tseytlin (1982), who felt that if migrators fed even a small amount at depth, then the net export caused by excretion would be slight. In the present model, the point at which no net flux will take place was found to occur when the levels of daytime feeding were at 18 % of those at night.

The active flux of carbon and nitrogen

It is evident that those factors affecting the dawn-dusk difference in carbon and nitrogen weight will also affect the magnitude of the active flux. The considerations outlined

above therefore also apply in this instance. However, the fact that the dawn-dusk difference was expressed in relative terms (i.e. percentage change), while the active flux is expressed in absolute terms (i.e. μg C or N m^{-2} d^{-1}), means that other factors will also be important here. Increases of a given magnitude in both the migrating biomass and the initial carbon and nitrogen weight of individuals resulted in increases in the active flux of the same magnitude. In terms of the migrating biomass, this linear function is easy to understand: more individuals swimming into the depths means that more material will be transported in their body tissues. Similarly, the more carbon and nitrogen that these individuals contain, the more material will be transported and the more will be metabolised and released into the water column. That is to say, larger individuals can cause a greater active flux due to the weight-specific nature of excretion and respiration. This is also the reason why the levels of nighttime feeding are important: more feeding through the night results in an increase in the carbon and nitrogen weight of individuals swimming to depth at dawn, and hence an increase in the amount of material available to be metabolised and released at depth.

Carbon and nitrogen growth

Growth was reduced as the amount of time spent at depth was increased (in those scenarios where no feeding occurred at depth), and increased as levels of feeding increased. Furthermore, because feeding levels were always assumed to be higher at the surface at night, the best growth was associated with time spent in the surface layer. However, given the large proportion of the day spent at depth at this time of year, it was also apparent that growth levels could be significantly increased by even low rates of feeding at depth.

A broader perspective of the export flux at BATS

For a broader perspective, it is interesting to consider the overall amounts of material that might be cycling through the Sargasso Sea as a whole (estimated surface area, 5.12 $\times$ 10^{12} m^2). Extrapolating the ^{14}C uptake rates measured during the BATS time-series programme in August/September 2000 (Figure 5.9) shows that, over a year in the Sargasso Sea, 0.8472 Pg C would be fixed into the surface waters by primary production. Similarly, the trap-measured passive sinking of particulate material (Figure 5.10) shows that 0.0279 Pg C would be removed from the surface 200 m. The model results showed that, in a year, up to 0.0015 Pg C could have been removed from the top 200 m of the Sargasso Sea via the NDVM of *P. xiphias* adults alone. This active flux equates to 0.18 % of primary production, and 5.54 % of the sinking POC flux at 200 m. All of these values are likely to be underestimates of the annual means, however, given that neither primary production, sinking fluxes, nor zooplankton migrating biomass are typically at their annual maxima in August and September.

According to the Energy Information Administration (URL: http://www.eia.doe.gov), a total of 6.198 Pg C (where 1 unit of carbon equates to 3.667 units of CO_2) were released into the atmosphere as CO_2 in 1998 (Sources: Petrol, 45 %; Coal, 35 %; Gas, 20 %). A quarter of this was produced by the United States alone, while other significant contributors included China (12 %), Russia (6 %), Japan (5 %), Germany (4 %) and India (4 %). From the calculations presented here, the Sargasso Sea would have been responsible for fixing 13.7 % of these global atmospheric carbon emissions via photosynthesis, for sequestering 0.5 % below 200 m via passive sinking, and for sequestering 0.02 % below 200 m via the NDVM behaviour of *P. xiphias* adults. Put another way, the NDVM behaviour of *P. xiphias* adults in the Sargasso Sea might have been responsible for mitigating 100 % of the CO_2 emitted by either Brunei or

Gabon in 1998, or perhaps 20 % of that emitted by Ireland. While these calculations are quite evidently pushing the boundaries of extrapolation, what they do highlight is that the NDVM behaviour of interzonal zooplankton is indeed capable of sequestering noticeable amounts of atmospheric CO_2 (and indeed other gases, such as NO and N_2O) in the ocean interior.

7.2.3 Discussion

While each of the factors analysed (carbon and nitrogen weight, levels of feeding, time spent at depth, migrating biomass) had an effect on the active flux, the two most influential were the amount of feeding at depth, and the migrating biomass. It is therefore particularly important in any study of the active flux to understand and quantify these two parameters accurately. Not shown in Table 7.1 were the relative contributions of defaecation, excretion and respiration to the active flux. DIC respiration was the most important contributor to the active flux of carbon (65 % of the daytime losses when not feeding), and DN excretion the only contributor to the active flux of nitrogen. That said, however, this model does not account for the defaecation of POM at depth of material ingested in the surface layer, a process which Schnetzer & Steinberg (2002a) showed to be potentially important at BATS (up to 20 % of the sinking particle flux at 150 m). Given the fact that metabolic rate processes are temperature-specific, one must also consider the environmental temperature. In this model, the effects of changing the environmental temperature were interesting and profound. Increasing the temperature of the surface layer reduced the magnitude of the active flux (e.g. a 3 °C rise reduced the active flux by 1.5 %), while the opposite was true for the deep layer (e.g. a 1 °C rise increased the active flux by 10 %). This represents a positive feedback mechanism to temperature increases caused by global warming: as greenhouse gases

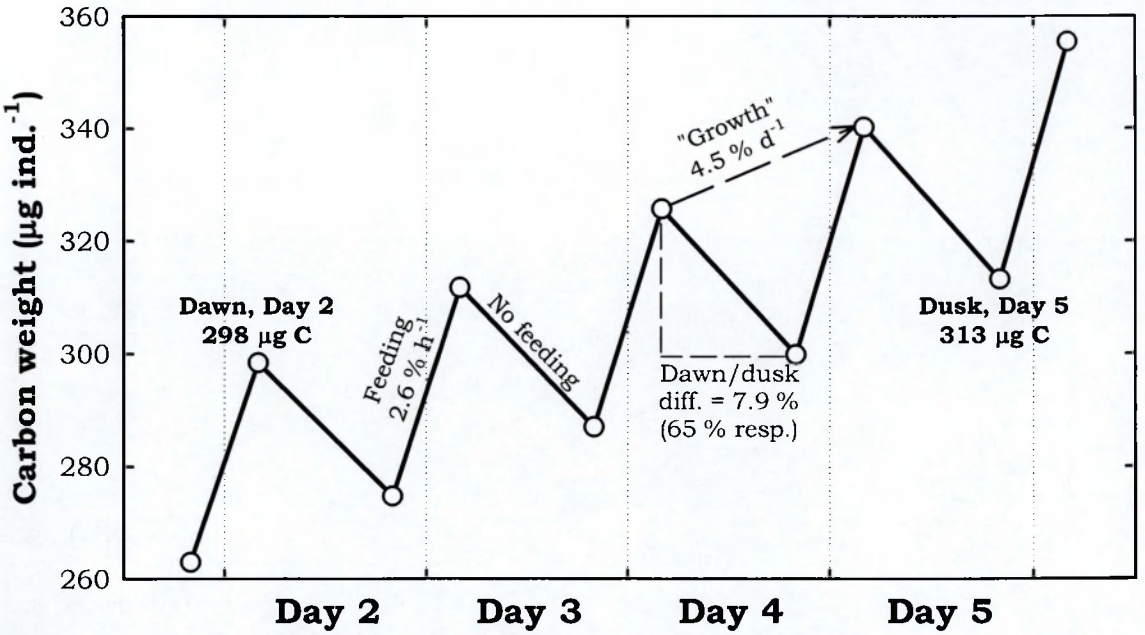

Figure 7.1 The modelled daily increases in the carbon weight of the interzonal diel vertical migrant *Pleuromamma* xiphias, which feeds at the surface at night and does not feed while at depth during the day.

increase in the atmosphere, sea-surface temperatures will rise, and the ocean will actually have a reduced capacity to sequester these gases via the active flux.

A final point of interest to be raised by this model is illustrated in Figure 7.1. In this situation, levels of feeding at night are high (2.6 % body C h^{-1}), while no feeding is occurring during the day. It is evident that the true active flux at any one time will be represented by the difference in the weight of an individual between dawn and dusk on the same day. This point is very important in the context of field sampling. In the sampling programmes undertaken during this study (chapters 4-6), and also during that of Hays *et al.* (1997b), samples were collected at dawn and dusk over the course of many days and the dawn and dusk samples grouped prior to comparison by ANOVA. However, as highlighted in Figure 7.1, this grouping could cause significant problems in the ability to quantify accurately the active flux. In this example, where levels of feeding remain constant over the 6 d period and net growth is occurring (i.e. feeding

inputs of carbon outweigh metabolic losses), the carbon weight of individuals at dawn on day 1 is actually less than those at dusk on day 5. Therefore, if we were to compare samples from these two days, we would arrive at an erroneous measure of the true active flux. It is apparent that this example does not account for variable levels of nighttime and daytime feeding over the study period, and we could vary the parameters in this model in endless ways. However, what is does highlight is that, in order to obtain the best measure of the active flux at a given time, one must compare the weight of migrants at dawn and dusk on the same day.

7.3 ZOOFLUX: a critical evaluation of the methodology

ZOOFLUX proposes that the total active flux at any given site can be quantified by knowing the diel change in the weight of the interzonal migrants and the specifics of their DVM behaviour (see chapter 2). While there is no reason why this should not still hold true in theory, the results presented in chapters 4-6 indicated that the execution of this rationale in the field is not as simple as first supposed. As an attempt to explain why this was the case, the following is a critical evaluation of the methodology, addressing the issues surrounding data collection, the accurate assessment of the dawn-dusk difference in body weight, and the fact that ZOOFLUX bases the diel changes occurring in the body weight of an individual on a model of average change.

7.3.1 Data collection

The collection of data will be subject to a variety of site-specific constraints, with the pros and cons of any given site relating to a variety of logistical, physical and biological considerations. Logistical constraints can be overcome, at least in theory, by increases in technology, personnel, and/or funding. Physical and biological constraints, on the

other hand, might prove to be too much for even the most well supported project. In this case, some advanced statistics may well prove to be the only way to extract useful information from any data available.

What is the likelihood that an active flux is occurring?

Of primary importance within any study of the active flux is to establish the presence or absence of interzonal migration. In chapters 4-6 it was shown that DVM was likely to have been occurring at most times of the year. It is therefore probable that there was a net transport of food-derived material from shallower waters to depth in most cases. However, considerations of the hydrographic regime at each site, both during this study and at other, unsampled, times of the year, showed that a barrier to mixing was not present during the winter months in Inchmarnock Water and at BATS, and not at all in Deep Cove. In these instances, even if an active flux of material was occurring, it may not have been significant in the sequestration of surface-ingested material. It must be borne in mind, however, that the lack of a pycnocline does not necessarily imply that material carried to depth will not be sequestered there for a length of time, as other hydrographic and biogeochemical factors may also be influential (e.g. storage in sediments). While worthy of consideration, the long-term fate of material released at depth by zooplankton migrants falls outside the scope of this study and will not be discussed here.

While it was found that DVM was probably occurring in most cases, the details were not elucidated until some time after each sampling cruise, following time-consuming laboratory and data-based analysis. The lack of real-time information therefore meant that the depth and timing of net tows had to be decided based on a relative amount of guesswork in the field. It is difficult to collect individuals at the optimum times decreed

by ZOOFLUX, especially at sites where DVM behaviour does not conform closely to a dawn descent and a dusk ascent. Indeed, this has arisen as the most important practical consideration to deal with when undertaking field-sampling for ZOOFLUX. Detailed knowledge of the migrant community therefore appears to be vital before specimen collections for ZOOFLUX can be carried out, and real-time methods for monitoring the DVM behaviour of zooplankton would be extremely useful.

Obtaining data: logistical considerations

Assuming that an active flux will be occurring at a given site, the logistical feasibility of obtaining suitable data to quantify this flux must then be considered. One must first consider the site's accessibility and the resources available once there. In other words, can you get there (and how often), what can you achieve once you have, and how much will it cost? Given limitless finances and an ability to coax assistance from others (these often work hand in hand), there is no real limit to the study sites available and the types of sampling that one might carry out. We do, after all, have the ability to send people to the moon, or to the deepest depths of the ocean. It is therefore apparent that such logistical constraints are inherently financial, with the technology available for oceanographic research being driven primarily by consumer demand and therefore the amount of money and expertise available for dedicated research and development. Indeed, this issue was discussed during the Second Marine Zooplankton Colloquium (MZC2, 2001): "Therefore it is not so much a lack of ideas but inadequate methodologies and instrumentation that limits the pace of advances in understanding marine zooplankton".

Both Inchmarnock Water and Deep Cove were particularly suited to regular and relatively low-budget sampling due to their proximity to shore and the fact that the

water column was <200 m deep. At these sites, the 1 m opening/closing WP-2 net was certainly sufficient for the collection of copepods. However, the low numbers or absence of euphausiids in daytime net catches at both sites indicated that this net might well have been unsuitable for the quantitative sampling of faster swimming species with well-developed vision. This meant that, for Deep Cove in particular, where *N. australis* was a significant contributor to the migrant community, a different net system would have been preferable. Studies at BATS require higher budgets, given the greater distance from shore and the need for a more substantial, ocean-going research vessel and sampling gear capable of acquiring data from depths of 800 m or more. It was fortuitous that DVM was sufficiently synchronised to obviate the need for an opening/closing net (although it would certainly have helped to resolve the vertical distribution of the migrant community), thereby reducing the complexity of field sampling and the potential for equipment failure (as was the case with the MOCNESS in Inchmarnock Water in August 1999). Other sites might not be as conducive to such simple equipment requirements.

With money and personnel no object, the information obtained from the studies carried out here might have been improved. For example, a MOCNESS (or other multiple net system) could also have been deployed at BATS and in Deep Cove, and towed more frequently at all sites. This would have required a more substantial research vessel in Deep Cove, and sampling cruises dedicated solely to this study in all cases (particularly at BATS, where a large proportion of each monthly cruise is taken up by sampling for the BATS programme). More people processing the zooplankton samples might have improved sample numbers, potentially removing some of the statistical problems inherent with low numbers and high variability (but see below). Similarly, the acoustic dataset might have been improved with the deployment of more ADCPs over a

range of frequencies, the physical environment better resolved with the deployment of more instrumentation, and so on.

Obtaining data: biological considerations

There are, however, some constraints that may reach even beyond a golden wallet and a silver tongue. In some cases, even collecting vast numbers of samples, processing them with the utmost diligence and accuracy, or applying complex statistical procedures to the data, might be insufficient to tease out the diel signal necessary for ZOOFLUX to work. This relates to the behavioural and physiological variability of individuals within the migrating community, and, as such, is a biological issue that may not easily be overcome. Indeed, variability in the natural world is an issue that has faced biologists for quite some time. As Darwin wrote in *The Origin of Species* (1859), "No one supposes that all the individuals of the same species are cast in the very same mould". As well as going on to say "These individual differences are highly important for us, as they afford materials for natural selection to accumulate...", he also suggested "it is the most flourishing, or...dominant species – those which range widely over the world...and are the most numerous in individuals – which oftenest produce well-marked varieties".

The issue of variability was particularly pertinent with regards to the *Calanus* population in Inchmarnock Water, which exhibited a high degree of individual variability in both the magnitude and timing of DVM behaviour. Perhaps this marked variability relates, as Darwin's idea would suggest, to the fact that *Calanus* is both abundant and widespread throughout the whole of the North Atlantic Ocean. This indicates that certain species (i.e. those exhibiting less variability) are more conducive to the application of ZOOFLUX than others. As Shine & Bonnet (2000) discussed, it is

for this reason that "particular taxa can serve as 'model organisms' for some types of [ecological] question". A model organism for ZOOFLUX might be *P. xiphias,* which is easily identified and sorted, exhibits marked interzonal NDVM, and is relatively easy to make accurate measurements on. However, it is still unclear as to why a strong diel difference in carbon or nitrogen weight was not detected in *P. xiphias* in the present study. One explanation is that the relatively high degree of physiological variability observed between sampled individuals was due to the likelihood that the same population was not sampled at each time-point. This is a common problem in open-ocean zooplankton investigations. Furthermore, there were times at each study site when even an army of assistants would not have helped, for the migrants were simply not caught in any great numbers in the net tows. This could have been due to patchiness, net avoidance or generally low population concentrations at the time. This was an issue at various times at all three study sites, and one that must have been experienced at one time or another by anyone undertaking zooplankton collections.

As one might define model organisms for specific studies, one might also define model study sites. A model site for ZOOFLUX would be one which is readily accessible and sheltered, contains an isolated community of easily identifiable, abundant and relatively large interzonal migrants, is well stratified and is conducive to the rapid collection of samples (i.e. is relatively shallow).

Obtaining data: physical considerations

Having established that a site is financially feasible for sampling, one must then consider the weather. In unsheltered environments there will always be a point at which the sea conditions are too dangerous for net sampling to be carried out, or for moored instruments to be deployed and recovered, or even for the research vessel to be at sea.

This was certainly an issue in Inchmarnock Water, when net-deployments were severely curtailed during the winter months and the temporal resolution of the moored instruments had to be decreased so that the mooring could be left unattended for longer periods if necessary. The weather is also a factor at BATS, with the regular passage of hurricanes in the summer, and low pressure systems in the winter. In fact, many of the time-series cruises over the years have been cut short by the near passage of hurricanes. As the official R/V "Weatherbird II" t-shirt points out, sampling cruises in this region truly are "defying the triangle" on occasion. Only Deep Cove is free from such constraints due to its particularly sheltered aspect. The trick to successful sampling must therefore lie in the ability to obtain the necessary information even in heavy seas. For this reason, moored instruments might be seen to hold the greatest potential, since these may be effective well beyond the point at which net deployments are no longer tenable (as well as providing information on superior temporal scales). Environmental probes such as thermistors, pyranometers and ADCPs are all useful in providing background information concerning the active flux, but zooplankton specimens must still be collected in order to quantify this flux. The development of an autonomous, moored zooplankton-collecting device might provide one solution to this problem. However, it might also be worthwhile to explore the potential for accurately inferring the weight of individuals remotely and *in situ*. A similar philosophy is employed when inferring chlorophyll *a* concentration from fluorescence, for example.

7.3.2 Quantifying diel changes in the body weight of interzonal migrants

The measurement of the active flux using ZOOFLUX relies on the detection of a significant diel change in the weight of zooplankton vertical migrants, in this case, carbon and nitrogen weight. It is therefore apparent that any methodological process

that may influence the carbon and/or nitrogen weight of an individual will subsequently influence the ability to quantify the active flux. Such influential processes will include the stress to individuals of capture and handling, and the way in which samples are processed.

The stress of capture and handling

When zooplankton are collected in net tows, they become subject to unnatural conditions that are bound to influence their composition. Some species, such as *M. norvegica*, may actually benefit from this environment as they have been shown to feed actively in the cod-end (Lass *et al.*, 2001). Others will not fare so well, and will be subject to physical damage and stress. In either case, it is apparent that short tows with the most coarse-mesh nets possible for the target species will be preferable to minimise changes in captured animals. In the relatively shallow waters of Inchmarnock Water and Deep Cove, individuals captured with the WP-2 net did not spend more than a few minutes in the cod-end, while those captured with the MOCNESS did not spend more than ~15 min. It is therefore likely that stress was kept to a minimum at these sites. At BATS, however, individuals captured at 500 m during the day may have spent up to ~60 min in the cod-end, the stress from which is likely to have induced potentially significant elemental losses. However, time is not the only factor, and the species composition in the cod-end is also likely to have an effect. For example, during the summer months in the Clyde Sea, the high levels of 'jellies' (*Beroe* sp. and *Cyanea* sp., mainly) in the net catches caused a noticeable decline in the quality of the *Calanus* samples, with a large proportion found to be dead and/or associated with tentacles and mucus. In addition, the hydrographic changes that a net-caught individual is subjected to as the net is retrieved from depth are likely to influence its elemental composition. For

example, exposure of net-caught krill to the surface freshwater layer in Deep Cove may have caused an osmotic release of material. One way to avoid all of these problems might be to fix the animals somehow at the time of capture. A similar philosophy is employed with the Continuous Plankton Recorder (CPR), for example, and it would be useful to design a net system capable of collecting specimens in good condition and fixing them immediately at their *in situ* depth.

Similarly, stress would have been induced when decanting the contents of the cod-end into containers, and when handling live individuals during analysis. This would have been an issue at each of the study sites. The use of carbonated water (both carbonated seawater and soda water were used at various times) to anaesthetise the animals was one way of circumventing this problem, although it is unclear as to whether this itself may have caused elemental loss via osmosis or other processes. Platt *et al.* (1969), for example, found that exposure of zooplankton samples to distilled water did not cause any loss of material, whereas Omori (1978) found losses of 6 % C and 7 % N in samples of *Calanus sinicus*. However, as Williams & Robins (1982) pointed out, these losses may have been due to the fact that samples were rinsed under vacuum.

Changes during sample processing

Following capture, individuals must then be identified, sorted and prepared for elemental analysis. The amount of time taken from cod-end to preservation (freezing or drying), and the amount of manipulation of each sample (with pipettes or forceps), will correlate positively with the amount of material potentially lost through stress. At BATS, the merits of two methods of sample preparation were tested, namely measuring length and gut fullness immediately post-capture in the shipboard lab followed by freezing (the "onboard" method), versus instant freezing post-capture and making all

measurements at a later date (the "ashore" method). It was shown that the reduced amount of shipboard stress-time resulting from the ashore method provided a more realistic assessment of the *in situ* gut fullness, but that the freeze/thaw process which this method entailed later on may well have caused a loss of material and therefore an underestimate of the real dry weight and elemental composition (the influence of freezing is discussed further below). It is therefore difficult to advocate one method over another, and the choice of which one to use must be based on personal preference and the specific situation. For example, the ashore method may prove to be preferable in open-ocean swells, where delicate microscope-based analysis is likely to be awkward and where seasickness is often an issue, while the onboard method would be better suited to more sheltered environments. Furthermore, it would not prove too involved to generate statistically robust conversion factors for each method so that both could be used and interchanged.

It is possible that the number of individuals per tin capsule (= sample) might have influenced the measurements of dry, carbon and nitrogen weight. This could be due to the reduced accuracy of measurements (especially dry weight) made on single individuals, or the effects of calculating an individual weight as an average of more than one individual. This possibility was explored using least-squares linear regression analysis on the number of individuals per sample (X_i values) versus the directly-measured weight of size-normalised individuals (Y_i values). Table 7.2 shows that there were only two instances (out of 15) in which the number of individuals per sample had a significant influence ($b > 0$, ANOVA: $P \leq 0.05$). Therefore, it would appear in most cases that the number of individuals per sample was not the most important source of error in the accurate measurement of dry, carbon and nitrogen weight.

Y_i values	Regr. param.	*Calanus*						*P. xiphias*
		Jun'99	Aug'99	Oct'99	Dec'99	Mar'00	May'00	Aug'00
Dry weight	r^2	-	-	-	-	<0.01	0.02	0.01
	b	-	-	-	-	0.417	-1.85	-13.15
	ANOVA: P	-	-	-	-	0.735	0.417	0.309
Carbon	r^2	0.05	0.06	0.13	0.03	0.20	-	<0.01
	b	3.35	7.07	-2.44	12.84	6.42	-	3.57
	ANOVA: P	0.038 *	0.2	0.336	0.314	<0.001 *	-	0.515
Nitrogen	r^2	<0.01	<0.01	0.15	<0.01	<0.01	-	<0.01
	b	-0.09	0.17	-0.80	-0.27	0.03	-	1.02
	ANOVA: P	0.793	0.767	0.307	0.776	0.932	-	0.522

Table 7.2 The results of simple (least squares) linear regression analyses of the relationship between the number of individual zooplankton migrants per capsule (X_i) values, and the measured dry, carbon and nitrogen weight (Y_i) values. Regr. param. = regression parameters. Asterisks (*) denote where the slope of the line (*b*) was significantly different from zero (i.e. ANOVA: $P \leq 0.05$).

However, it is still apparent that there are relative pros and cons to the measurement of individuals versus groups of individuals. While the accuracy of measurements will be improved with more individuals per sample, this does mean that individual variation will not be directly measured, resulting in the loss of potentially important ecological information. Indeed, where there were sufficient sample numbers for comparison, it did appear that the coefficient of variation was highest in those samples comprising fewer individuals. For example, the nitrogen weight of size-normalised (2.18 mm-long) *Calanus* collected from Inchmarnock Water in June/July 1999 showed the following variation:

1 individual per sample: $V = 26.6$ %

2 individuals per sample: $V = 18.1$ %

3 individuals per sample: $V = 17.2$ %

4 individuals per sample: $V = 13.2$ %

Therefore, a balance must be struck between obtaining sufficiently accurate measurements (dependent on the mass of the samples and the accuracy of the measuring equipment) and obtaining sufficient information on the true biological variation (most evident when measuring individuals).

The freezing of samples prior to drying may have also affected their dry, carbon and nitrogen weights. This process was carried out on all samples from Inchmarnock Water and BATS, but not on those from Deep Cove which were dried directly within ~40 min of capture. The effects of freezing were not studied here, but Williams & Robins (1982) have shown, from their studies on *Calanus helgolandicus* from the Celtic Sea, that this form of preservation is likely to disrupt membranes and internal tissues, allowing the escape of body fluids following thawing. Indeed, they found a 57 % reduction in the dry weight of adult females following thawing, a 48 % reduction in carbon, and a 60 % reduction in nitrogen. They attributed the proportionately greater loss of nitrogen, as have other researchers with other zooplankton species (e.g. Hopkins, 1968; Beers, 1976; Champalbert & Kerambrun, 1979), to the disproportionate loss of proteinaceous material and free amino acids. This disproportionate loss was also evident from the methodology comparison conducted during the present study at Hydrostation S. However, this freeze/thaw-related loss of material would not have been an issue when thawed samples were already contained in tin capsules: the escaped body fluids would have remained in the capsules, and therefore still been included when the measurements of dry, carbon and nitrogen weight were made. This was the case in all samples processed via the onboard method (i.e. all *Calanus* from Inchmarnock Water). However, those processed via the ashore method (i.e. all *P. xiphias* and krill at BATS) were thawed in petri-dishes before being placed in capsules, meaning that subsequent measurements of dry, carbon and nitrogen weight may well have been underestimates.

Freeze-drying (lyophilisation) is more gentle than oven drying (Postel *et al.*, 2000), and may therefore prove more suitable for obtaining the most accurate dry, carbon and nitrogen weight measurements for ZOOFLUX. While this processing method was not tested during the present study, Omori (1978) found that dry, carbon and nitrogen weight measurements were 2 % higher in freeze-dried samples compared to those dried in an oven. Similarly, Fudge (1968) found 3 % more lipids and 1 % more protein, chitin and ash ('ash' = all inorganic substance).

When making dry weight measurements, there are also potential problems with the hygroscopic nature of dried samples. That is to say, dried samples may increase in mass during the weighing procedure due to the uptake of moisture in the air. During this study, care was taken in all cases to reduce this uptake to a minimum. Samples were left for at least 24 h in a desiccator to adjust to ambient temperature prior to weighing, and, during the weighing procedure, individual samples were exposed to the air for no longer than 10 s at a time. Furthermore, each of the balances used were housed in a chamber in which a sachet of silica gel was included to reduce further the atmospheric moisture content. Lovegrove (1966) found that dried zooplankton samples increased in mass by 2 % after 5 min exposure to the ambient atmosphere, while Båmstedt (1974) found a 3 % increase after 20 h. From this, Postel (1990) suggested that these increases could be reduced to 0.05 % by weighing samples in a room with low humidity, not exceeding 30 s exposure to the air, and using samples $\leq$300 mg (since, presumably, larger samples absorb more moisture). Furthermore, Postel *et al.* (2000) advocated the use of balances with closeable weighing chambers containing sachets of desiccant material. Given these considerations, it would be fair to say that all possible precautions were carried out with the dried samples in this study.

7.3.3 Statistical analysis

Due to the destructive nature of sampling (i.e. having to combust samples to measure their carbon and nitrogen weights), it is impossible to monitor the same individual over the diel cycle. ZOOFLUX in practice is, therefore, a statistically-based procedure for quantifying the active flux, because it bases the diel changes occurring in the body weight of an individual on a model of average change. That is to say, measurements of the weight of different individuals collected at different times are assumed to represent the temporal changes in weight that would be occurring to the same individual. In terms of the analogy given in section 2.1, this would be the same as counting the money in one person's pocket on entering a shop and in a different person's pocket on leaving, and using this difference to represent the average amount of money that each person spends while inside. The normalisation procedure aims to ensure that the same 'type' of person is being measured: one would not expect a student to spend as much as a professor, for example, so one needs to define an average 'spending power'.

This averaging may well prove to be a significant source of error, particularly if levels of individual variability in physiology (spending power) are high. One way in which this issue was addressed was by size-normalising the raw carbon and nitrogen weight measurements. However, this raises the question of what is the best measure of 'size' to use. Is it best to use the parameter that shows the least variability (i.e. length in this case), or that which shows the most (i.e. dry weight in this case)? Using the least variable parameter means that the size-normalised data will include more of the original variability inherent in carbon and nitrogen weight. This would therefore preserve a greater amount of ecological information in much the same way as measuring individuals as opposed to groups of individuals. It does, however, mean that any real diel changes are more likely to be statistically masked by variability (i.e. there would be

a greater probability of making a Type II statistical error). Similarly, while size-normalisation using dry weight is less likely to cause diel changes to be masked, there is a greater risk that these diel changes would not have been real (i.e. there would be a greater probability of making a Type I statistical error). Again, a balance must be struck between the levels of variability in each of the measurements, and the practicality of obtaining accurate measurements of these parameters.

The statistical assessments of the success of this model of average change (see section 2.3.8) at each of the three study sites (sections 4.3.4, 5.3.5, and 6.3.3) showed that low sample numbers (n, expressed as the arithmetic mean of the number collected at both dawn and dusk) and high variability in the size-normalised carbon and nitrogen data (V, expressed as the mean of the coefficients of variation at both dawn and dusk) were at least partly responsible for the inability to detect significant (ANOVA: $P \leq 0.05$) daytime decreases in the body weight of individuals: n ranged from 5 to 62, and V from 7 to 24 %. Of course, it is also possible that, at least in some cases, daytime feeding would have been occurring at such a level that there was no daytime decrease to detect. The fact that, in 13 out of 24 comparisons, a dawn-dusk increase in body weight was found, even at times when daytime feeding was thought to be low (see Tables 4.11, 5.8 and 6.8), would suggest that the model of average change was not effective in picking up a dawn-dusk decrease at times when one would have expected it to exist. Furthermore, the fact that the probability of having made a Type II error ($\beta \times 100$) was high (62-95 %) in every one of the 10 out of 24 comparisons where the dawn-dusk difference was not significant (ANOVA: $P > 0.05$) lends weight to the idea that the present field-tests of ZOOFLUX were ineffective due to low n and high V. In many cases, the high V meant that unfeasibly high numbers of samples would have been required in order to detect diel changes: a feasibly obtainable sample size might be 50

samples per time point, while the statistical calculations suggested that up to 45,000 samples would have been required in one case (range 12-45,000). Both the low n and the high V also meant that the ANOVA tests were not sensitive enough to detect biologically feasible differences: an individual might be expected to lose ~10 % of its body weight during a daytime period of reduced feeding, while the detectability of the comparisons carried out during the present study ranged from ~10 to ~50 %.

7.4 A synopsis and suggestions for future research

As Steinberg *et al.* (2002) wrote, "estimated rates of active transport will be conservative until all the potential fluxes of dissolved and particulate, inorganic and organic, matter are determined". As discussed in section 1.2.4, nearly all of these "potential fluxes" (in terms of carbon and nitrogen) have now been investigated, such that we might expect to have a fair understanding of their relative importance in the sequestration of material in the ocean's interior. So, after more than a decade of research since the first focused study of Longhurst & Harrison (1988), what is the status of our present knowledge of the active flux, and where do we need to go from here?

7.4.1 How much material are vertical migrants sequestering in the ocean interior?

Methods used in active-flux studies

Only a handful of studies have provided values for the active flux of carbon and/or nitrogen in the marine environment. These are listed (refs. 1-16) in Table 7.3. The technique in each case has been to use various assessments of the biomass and vertical distribution of the zooplankton community in conjunction with various assessments of the rates of important processes (i.e. excretion, respiration, defaecation, feeding and mortality) in order to estimate the active flux of either carbon or nitrogen across a given

depth boundary. The philosophy of the present study is similar in that it assessed the biomass and vertical distribution of the zooplankton community, but different in that no rate process assessments were required, only diel changes in the body weight of individual migrants.

Conventional net tows were employed in each of those cases where zooplankton and/or micronekton specimens were directly collected for analysis (refs. 1, 3-5, 7-13, 15, 16). Roughly half of these studies undertook depth-discrete tows (refs. 1, 3-5, 8, 10), while the rest undertook depth-integrated tows (refs. 7, 9, 11-13, 15, 16). The net-caught samples were size-fractionated in many cases (refs. 3, 4, 7, 8, 10, 13), and variously used to estimate zooplankton biomass in terms of displacement (ref. 1), wet weight (refs. 3, 4), dry weight (refs. 7, 10, 13), ash-free dry weight (ref. 10), or carbon/nitrogen weight (refs. 7, 8, 13). Less than half of these studies identified specific taxa within the net tows (refs. 1, 3, 5, 9, 10), while a few assessed *in situ* feeding from the gut chlorophyll content of individuals caught at various times during the diel cycle (refs. 3, 8). In one study (ref. 1), *in situ* observations and collections of the plankton were made by SCUBA diving, while in another (ref. 3), a 37 kHz echosounder was used to monitor the migrant community. Many of the studies obtained metabolic data from their own shipboard incubation experiments (refs. 1, 3, 4, 7-10, 12, 15, 16), while a number used data already available in the literature (refs. 1, 8, 11, 13). Only one study (ref. 3) made direct measurements of the light regime, while a number employed measurements of the passive particle flux and/or the levels of primary production with which to compare their active flux assessments.

Pathway	Location	Date	Depth (m)	Flux: mg m^{-2} d^{-1} (% of sinking flux)	Reference
Respiration					
DIC	7 oceanic	Various	Various (D2)	3-106.6 (12.9-52.8)	4
	Sargasso Sea	Mar/Apr'90	150	6-41 (18-70)	8
	Equatorial Pacific	Sep/Oct'94	122-144	n/a (4.2-9.4)	10
	Equatorial Pacific	Various	120	4.2-7.3 (18.4-25.4)	11
	Sargasso Sea	Various	150	1.5 (6)*	12
	Subtrop. N Pacific	Various	155	2.6-4.4 (10.7-18.1)	13
Excretion					
DIN	9 oceanic, 1 coastal	Various	30-130	0.4-16.5 (1.8-63.5)	2
	N Sargasso Sea	Sep'88	150	0.72 (7.7)	3
	N Atlantic	Apr/May'89	?	31 (26)	7
	Sargasso Sea	Mar/Apr'90	150	2-5 (17-82)	8
	Equatorial Pacific	Sep/Oct'94	122-144	3.6-3.7 (9-40)	10
	Subtrop. N Pacific	Various	155	0.5-0.8 (15.9-25.4)	13
	Sargasso Sea	Various	150	0.4 (8.5)*	16
DOC	Sargasso Sea	Various	150	0.5 (2)*	12
DON	Sargasso Sea	Various	150	0.2 (4.3)*	16
Defaecation					
POC	NW Atlantic	Aug'75	500	8.5-137 (n/a)	1
	NE Atlantic	?	?	0.9-21 (?)	6
	S Georgia	Feb'94	45-65	0.4 (n/a)	9
	Sargasso Sea	Various	150	<0.1-5.3 (<0.1-18)	15
PON	Sargasso Sea	Various	150	<0.1-1.0 (<0.1-20)	15
Mortality					
POC	NW Atlantic	Aug'75	500	3.6 (n/a)	1
	N Atlantic	Winter	500	0.75 (<0.1)†	5
	Equatorial Pacific	Various	120	2.9-5.4 (12.6-18.8)	11
	Southern Ocean	Summer	500	4.7-25.5 (114)†	14

Table 7.3 Previous studies of various pathways contributing to the active flux of either carbon or nitrogen. * = mean values only (where a maximum but no minimum value was provided). All values represent fluxes due to DVM behaviour, except †, which represent fluxes due to SVM/OVM behaviour (which also take into account DIC respiration). References: (1) Wiebe *et al.*, 1979 (2) Longhurst & Harrison, 1988 (3) Longhurst *et al.*, 1989 (4) Longhurst *et al.*, 1990 (5) Longhurst & Williams, 1992 (6) Morales *et al.*, 1993 (7) Dam *et al.*, 1993 (8) Dam *et al.*, 1995 (9) Atkinson *et al.*, 1996 (10) Le Borgne & Rodier, 1997 (11) Zhang & Dam, 1997 (12) Steinberg *et al.*, 2000 (13) Al-Mutairi & Landry, 2001 (14) Bradford-Grieve *et al.*, 2001 (15) Schnetzer & Steinberg, 2002a (16) Steinberg *et al.*, 2002.

Results and global extrapolations

By combining the data presented in Table 7.1, one might tentatively begin to estimate the total active fluxes of carbon (DIC + DOC + POC) and nitrogen (DIN + DON + PON). According to these data, the total active carbon flux across the density discontinuity ranges from 3 to 270 mg C m^{-2} d^{-1}, equating to 8 to 204 % of the concurrently-measured sinking fluxes of POC. Similarly, estimates of the total active nitrogen flux range from <1 to 32 mg N m^{-2} d^{-1}, equating to 6 to 106 % of the sinking fluxes of PON. To assess the global significance of these values, the assumption of Longhurst *et al.* (1990) will be followed, namely that the permanently stratified region within which the active flux might be significant ranges from 50 °N to 50 °S. As these authors showed, this region comprises 79.3 % of the area of the ocean, which, from the total ocean-area estimate given in section 1.1.4, approximates to 278×10^{12} m^2. The estimates given above would therefore suggest that, between these latitudes, zooplankton DVM causes the removal of up to 0.075 Pg C, and up to 0.009 Pg N every year into the ocean interior. In terms of carbon, this would equate to a maximum of 2.7 % of the global estimates of particle flux for the same region (see Discussion in Longhurst *et al.*, 1990), and would mitigate a maximum of 1.2 % of annual global anthropogenic CO_2 emissions (based on data for 1998, see section 7.2.2). Recalling the model extrapolations presented in section 7.2.2, the estimated annual active flux of 0.0015 Pg C caused by the DVM of *P. xiphias* adults over the whole of the Sargasso Sea (= 1.8 % of the ocean area between 50 °N and 50 °S) would equate to 2 % of the estimated annual global active carbon flux given above. While there are obviously issues with simply combining the various literature estimates in this way and extrapolating a globally-generalised range for the total active flux, given that each of these studies was site, time and/or species specific, what it does suggest is that the

active flux is certainly a process worthy of inclusion within considerations of global biogeochemical cycles. This recognised potential for inaccuracies in making large-scale extrapolations from spatially- and temporally-restricted studies, however, is reflected in the fact that these global estimates are an order of magnitude lower than those of Longhurst *et al.* (1990) who considered the respiratory flux alone. Indeed, this echoes the point made by these authors that these estimates cannot be taken "as serious revisions of the global carbon budget. Rather, they are evidence that the consequences for the carbon budget of massive daily translocations of biota in the open ocean are at least predictable with regard to their sign". After more than a decade of research, it is testament to the complexities inherent in studies of marine zooplankton that we are still unable to quantify the global significance of the active flux with any more confidence than merely to predict its sign, although the indications are that, globally, it will only be a relatively small fraction of the passive flux. Therefore, the time has come when we need to take stock of past findings, and decide how best to proceed so that we might obtain a more accurate global picture of the active flux.

7.4.2 Future research: where do we go from here?

"Future marine zooplankton research: a perspective"

A recognised need by the international scientific community to reflect on past zooplankton research in general, and to define directions for future work, resulted in the first Marine Zooplankton Colloquium (MZC1, 1989) in April 1988, leading to a second Marine Zooplankton Colloquium in February 1999 (MZC2, 2001). It is certainly interesting, if a little disconcerting, that, "despite more than 100 y of research on these organisms, our knowledge of their ecological function in their natural environment has increased only modestly", a fact attributed less to "a lack of ideas" and more to

"inadequate methodologies and instrumentation" (MZC2, 2001). MZC2 briefly reviewed the progress made since MZC1, showing that, while significant advances in knowledge have been made in several areas, it would appear that we are still constrained by methodological and technological inadequacies. In addition, three further issues were raised, one of which (issue 3: understanding better the role of zooplankton in global biogeochemical cycles) is of obvious relevance to the present study. It was suggested that biogeochemical considerations have been "inadequately addressed in highly productive regions such as ocean margins", and that "the main roles and contributions of key dominant species have in most cases received inadequate attention" (MZC2, 2001). Proposed steps to address this issue included using new biomarkers to trace the fate of organic matter, determining the production mechanisms and composition of DOC, and developing empirically driven biophysical models. For future zooplankton research in general, three broad steps were suggested during MZC2: (1) continuous long-term observations of zooplankton and their physical, chemical and biological environment, accompanied by (2) *in situ* rate quantifications of important processes (feeding, growth, mortality etc.), and paralleled by (3) the development of interdisciplinary models.

Future active-flux research: some recommendations

Continued efforts to understand and quantify the active flux over a range of areas and seasons are therefore justifiable. One particularly salient point that has arisen is this: any studies of the zooplankton, particularly those that deal with their ecology, require a broad-ranging and multi-disciplinary approach. Zooplankton are an integral part of a dynamic and complex environment, and zooplankton studies, the present one being no exception, must also consider an often overwhelming array of physical, chemical and

biological factors in order to provide a perspective to the results. It is certainly apparent that, in order to understand and quantify the active flux, we must first understand and quantify the movements and motivations of individuals before we can begin to consider the significance of their life processes.

So, which methods should be used in future active-flux studies? While it is true that the ZOOFLUX technique did not yield values for the total active fluxes of carbon and nitrogen during the present study, it has been shown that this apparent 'failure' was due, not to any flaws in the actual concept, but in fact to a variety of logistical, biological and statistical factors, some of which might well be mitigated with the benefit of hindsight and a carefully-designed sampling programme. Indeed, such constraints will also have been inherent to varying degrees in the approaches taken by those other investigators who have attempted to quantify the active flux: certainly both the ability to understand the dynamics of the zooplankton community and to quantify their metabolic rate processes will have been constrained by various factors. One might, therefore, advocate the use of the ZOOFLUX technique in future studies, given its *in situ* and holistic approach, but only if the various issues raised during the present study can be adequately addressed. An outline of how we might proceed is given in Table 7.4. This is intended as a field-sampling guide and, as such, makes no recommendations as to how the data generated might be used. Such empirically derived data would certainly be of use in the development and parameterisation of bio-physical models, which, in turn, can be used to understand ecological mechanisms and to predict the functioning of an ecosystem under a range of hypothetical scenarios. The development of such models has been widely advocated in the scientific community, particularly in recent years (see e.g. MZC2, 2001). As a step in this direction, the following chapter presents a nitrogen-based ecosystem model, modified to include the effects of zooplankton DVM.

Table 7.4 Recommended methodology for future studies of the active flux in the marine environment.

Step	Procedure	Details
1	Identifying study sites and target species	Firstly, both model sites and model species need to be defined, based on the considerations discussed in section 7.3.1, and with a view to the global applicability of the results. In terms of sites, it has been suggested that future active-flux studies should be made "along gradients of latitude and productivity, similar to that of Ikeda (1985)...to establish global generalisations" (issue 3: MZC2, 2001). In terms of zooplankton taxa of significance, it was also suggested that "selection of 'target' species should certainly require that they be major players in a significant ecosystem", or that "other criteria might include amenability to culture and information presently available in the literature" (issue 2: MZC2, 2001).
2	Defining the scope of the sampling programme	Ideally, studies should aim to be multi-disciplinary (i.e. should consider physical, chemical and biological parameters of relevance to the zooplankton community), and look towards making longer-term (i.e. seasonal), higher-resolution (both spatial and temporal) measurements if possible.
3	Defining the movements and motivations of individuals	The first task at a given study site must be to define the community dynamics as thoroughly as possible in terms of species composition, concentration and vertical distribution, and especially in terms of the movements of individuals across a density discontinuity. Indeed, one of the main reasons that the active flux could not be satisfactorily quantified in the present study was the lack of a detailed understanding of the migrant community at the time of specimen collection: the uncertainties as to whether samples consisted of individuals just as they crossed through the pycnocline certainly increased the amount and complexity of data analysis and interpretation that was required, hence the often lengthy discussions throughout. Recall the model considerations in section 7.2, which suggested that the migrating biomass is one of the most important parameters to measure accurately in an active-flux study. This is not an insignificant undertaking, and quite possibly represents a separate study in itself, especially if wishing to understand seasonal changes in the zooplankton community. CTD casts and conventional net tows will continue to be of use in this regard, while techniques such as acoustics and optics promise to provide invaluable additional information on time and space scales not achievable with net tows. The issues concerning the accurate interpretation of vertical migration have been comprehensively discussed by Pearre (1979a, in press). Only once this has been done can the final choice of target species be made, and the collection of specimens be accurately directed. Without this knowledge, one runs the risk of collecting samples that are inappropriate for the quantification of the active flux according to ZOOFLUX.
4	Confirming the ability to detect significant changes in the field	The chosen target species must first be assessed as to their suitability for the application of ZOOFLUX. Specifically, laboratory-based incubations on field-sampled individuals should be carried out to show whether or not statistically significant temporal changes (ANOVA: $P \leq 0.05$) in the mean weight of individuals can be detected under a range of feeding regimes. Indeed, as shown in the present study, if such changes cannot even be detected in starved individuals, then there is little use in attempting to detect changes in the field. Furthermore, *in situ* zooplankton incubations, such as the grazing studies carried out by Roman *et al.* (1993), promise to yield the most useful information regarding the important rate processes of interzonal migrants. The model considerations in section 7.2 suggested that feeding at depth was an important parameter to quantify accurately, and that DIC respiration and DN excretion were the most influential metabolic processes in the active fluxes of carbon and nitrogen, respectively.
5	Collecting ancillary data	Assuming that further sampling is deemed feasible (following step 4), a greater understanding of the ecology of the target interzonal migrants, and hence the active flux, will only be possible if ancillary data are also collected. That is to say, "a comprehensive understanding of a zooplankton species' existence can only be accomplished if those parameters (including other zooplankton taxa) affecting the species, and being affected by the respective species, are included in the assessments" (MZC2, 2001). Useful parameters to measure include irradiance (including both ambient and downwelling light), hydrography, primary production, phyto- and zooplankton species composition and vertical distribution, sinking particle flux, and other species which may be acting as either predators or competitors.

Table 7.4 (continued)

Step	Procedure	Details
6	Collecting migrant specimens	Having defined the specific movements of interzonal migrants as comprehensively as possible (step 3), it should then prove possible to collect migrants at the correct depths and times as they pass both up and down through the density discontinuity. The most practical method of collection is via net tows. Nets should be fitted with the coarsest mesh possible for the target species, to reduce the stress of capture. The development of a net system whereby samples can be fixed in good condition at their residence depth, yet still remain viable for biometric measurements, would be of great benefit in reducing the potential changes associated with time spent in the cod-end. Tows at other times can also be accurately directed to the depth strata in which migrants are known to be residing. If it was not possible to carry out step 3, or if the target species had been found to undertake interzonal migrations that are more complex than the simple pattern of NDVM (as observed with *Calanus* during the present study, for example: see section 4.3.3), it is advisable to carry out a series of depth-discrete net tows at each sampling time-point in order to 'hedge your bets'. Real-time techniques for monitoring the vertical distribution of the community, such as acoustics, would be of great benefit at the specimen-collection stage in confirming that movements are conforming to the patterns previously described. Furthermore, the development of an autonomous, moored zooplankton collecting device would be useful in allowing higher-resolution collections to be made, and perhaps also at times when the sea-state is too rough for net tows. Similarly, it might be useful to explore the possibility of inferring the body weight of individual migrants remotely and *in situ*.
7	Processing migrant specimens	The ultimate aim is to define the length, gut fullness and dry, carbon and nitrogen weight of individual migrants at different stages of their known DVM cycle. The issues surrounding sample processing were discussed in section 7.3.2. The choice of 'onboard' or 'ashore' processing will depend on the sea-state, the former being feasible on calm days, and *vice versa* (see section 5.3.4). A separate study to define conversion factors between these two methodologies would be extremely useful. The number of individuals per 'sample' will depend on the variability of the target species as quantified in step 4: it is best to measure individuals (to describe as much of the true ecological situation as possible), but this is only practical when variability is below a certain threshold. Wherever possible, the measuring instruments used should be precise enough to provide data with $\geq$95 % accuracy (see Equation 2.2). Where the 'onboard' method is employed, one should avoid freezing the samples if possible. Where the 'ashore' method is employed, one cannot avoid freezing the samples. It is therefore necessary to define by experiment the amount of material that is potentially lost following thawing in a petri-dish. For samples from both methods, freeze-drying is preferable to oven drying when preparing samples for dry, carbon and nitrogen weight measurement. When dry weighing, the guidelines provided in section 7.3.2 should be followed to minimise the hygroscopic uptake of moisture.
8	Data analysis	As highlighted in section 7.2.3, it is important to use only samples collected on the same day when estimating the diel changes in the weight of individuals. The carbon- and nitrogen-weight data can be usefully size-normalised to both length and dry weight to ascertain the effect of each of these measures of size on the ability to detect the necessary diel change in weight for ZOOFLUX to work. In order to increase the sample size, and therefore the chances of detecting smaller diel changes in body weight, one might use more than one net when making net tows at any given time, and/or have more people available to process the catch once landed. If variability in the carbon and nitrogen data is not sufficiently reduced following size-normalisation, then individuals also need to be grouped in samples. It may be that, in those species where individual variability in carbon and nitrogen weight at any one time is high, significant (ANOVA: $P \leq 0.05$) diel changes in body weight will not be detectable unless a way is found of non-invasively measuring the same individual over time.

8

A SEASONAL MODEL OF THE VERTICAL EXPORT OF NITROGEN IN THE SARGASSO SEA

8.1 Introduction

The extensive collection of field data is key to gaining an understanding of the carbon cycle. However, in order to predict its functioning in the future, one also needs to synthesise and model these data. As Doney *et al.* (2002) explained, "The field data collected as part of the international Joint Global Ocean Flux Study (JGOFS) provide an unprecedented view of marine biogeochemistry and the ocean carbon cycle". It is thanks to the hard work of those involved in a variety of field studies over the last decade, and the availability of their data to the scientific community, that we are now in a position to develop and refine models which will be able to predict the future levels of greenhouse gases such as CO_2. For example, field data are available from two time-series studies (the Hawaii Ocean Time Series, HOT: Karl & Lukas, 1996; the Bermuda Atlantic Time-Series Study, BATS: Michaels & Knap, 1996), the joint JGOFS/WOCE (World Ocean Circulation Experiment) global CO_2 survey (Wallace, 2001), and a number of regional process studies in the North Atlantic (the North Atlantic Bloom Experiment, NABE: Ducklow & Harris, 1993), the Equatorial Pacific (EqPac: Murray *et al.*, 1995), the Arabian Sea (Smith *et al.*, 1998) and the Southern Ocean (AESOPS: Smith *et al.*, 2000). Over 100 scientists are now involved in the final phase of the JGOFS programme, the Synthesis and Modeling Project (SMP: Doney *et al.*, 2002).

Marine ecosystem models have been used in oceanography for over 50 y (e.g. Riley, 1946; Steele, 1958, 1974), but began to receive increased attention ~20 y ago by investigators such as Evans & Parslow (1985), Frost (1987), Fasham *et al.* (1990) and Moloney & Field (1991). For example, Fasham *et al.* (1990) presented a seasonal, nitrogen-based model of plankton dynamics in the oceanic mixed layer at Hydrostation S off Bermuda (32.2 °N, 64.5 °W). The objective of this model was "to learn more about the role of oceanic biology in regulating the atmospheric CO_2 content". It was

posited that, since nitrogen is typically the limiting nutrient in marine primary production, then an understanding of the dynamics of this element is needed before we can begin to understand the oceanic carbon cycle. It was also advised that the dynamics of planktonic ecosystems are best understood by considering the seasonal cycles of plankton, nutrients and physics. Since this time, a great deal of progress has been made with regard to modelling the oceanic carbon cycle (e.g. Sarmiento *et al.*, 1990, 1993; Doney *et al.*, 1996; Fasham, 1995; McClain *et al.*, 1996; Six & Maier-Reimer, 1996; Chai *et al.*, 1996; McCreary *et al.*, 1996; Cox *et al.*, 2000; Dutkeiwicz *et al.*, 2001; Aumont *et al.*, 2002). To date, however, little attention appears to have been directed to modelling the active flux. This is perhaps because of a generally held belief that this is a relatively insignificant process within the cycles of biogeochemically important elements.

With this in mind, a new version of the Fasham *et al.* (1990) mixed-layer model is presented here, modified to include contributions by interzonal migrant zooplankton and a more detailed description of the sources and fates of nitrogen below the mixed layer. Like the original, the new model is again based on data from the Sargasso Sea off Bermuda. For convenience, the original model is referred to as M1, and the modified model as M2. M2 was built and run using the software package Powersim Constructor. The objectives of M2 at this stage are twofold: (1) to demonstrate a way in which zooplankton DVM can be included in biogeochemical ecosystem models, and (2) to investigate the significance of zooplankton DVM on the seasonal cycle of nitrogen-export from the surface mixed layer. The seasonal aspect to M2 builds upon the sensitivity analysis presented in section 7.2, in which the dynamics of the active flux in the Sargasso Sea were modelled on a daily timescale. M2 stems from an original nitrogen-phytoplankton-zooplankton-detritus (NPZD) model developed by K.J. Flynn

(KJF) at the University of Wales, Swansea, and to whom many of the modifications presented here must also be credited. To facilitate comparison with M1, this account follows the same structure as that of Fasham *et al.* (1990), with the aim being to highlight the addition of new concepts, equations and results to the original model. For those aspects in which M1 and M2 are the same or similar, reference is made to the original model to avoid repetition.

8.2 The model structure and equations

8.2.1 Model structure

As Fasham *et al.* (1990) discussed, models describing mixed-layer elements such as carbon or nitrogen in terms of compartments (pools, or state-variables) typically assume "that the mixed layer can be considered biologically homogeneous". That is to say, the compartment in question is assumed to have the same volumetric concentration throughout the mixed layer at any given time. Furthermore, they suggest that this assumption is probably "robust" except in areas, such as the northeast Atlantic, where the mixed layer can become so deep in the winter (up to 500 m: see Fig. 9 in Longhurst & Harrison, 1989) that concentrations may begin to show variation with depth. In M2, the inclusion of deep-layer compartments adds a new dimension to this assumption. For practical purposes, the volumetric concentration of a deep-layer compartment can be assumed to be uniform only to a depth of as much again as the mixed layer above it at that time. Below this depth, the concentration is assumed to be zero.

In M2, mixed-layer nitrogen is envisaged to exist in six compartments, and deep-layer nitrogen in four (Figure 8.1). For the sake of simplicity, dissolved nitrogen (N) is modelled as a single term in both the mixed and the deep layer (N_a and N_b, respectively), given that the focus of M2 is now on zooplankton DVM, and not primary

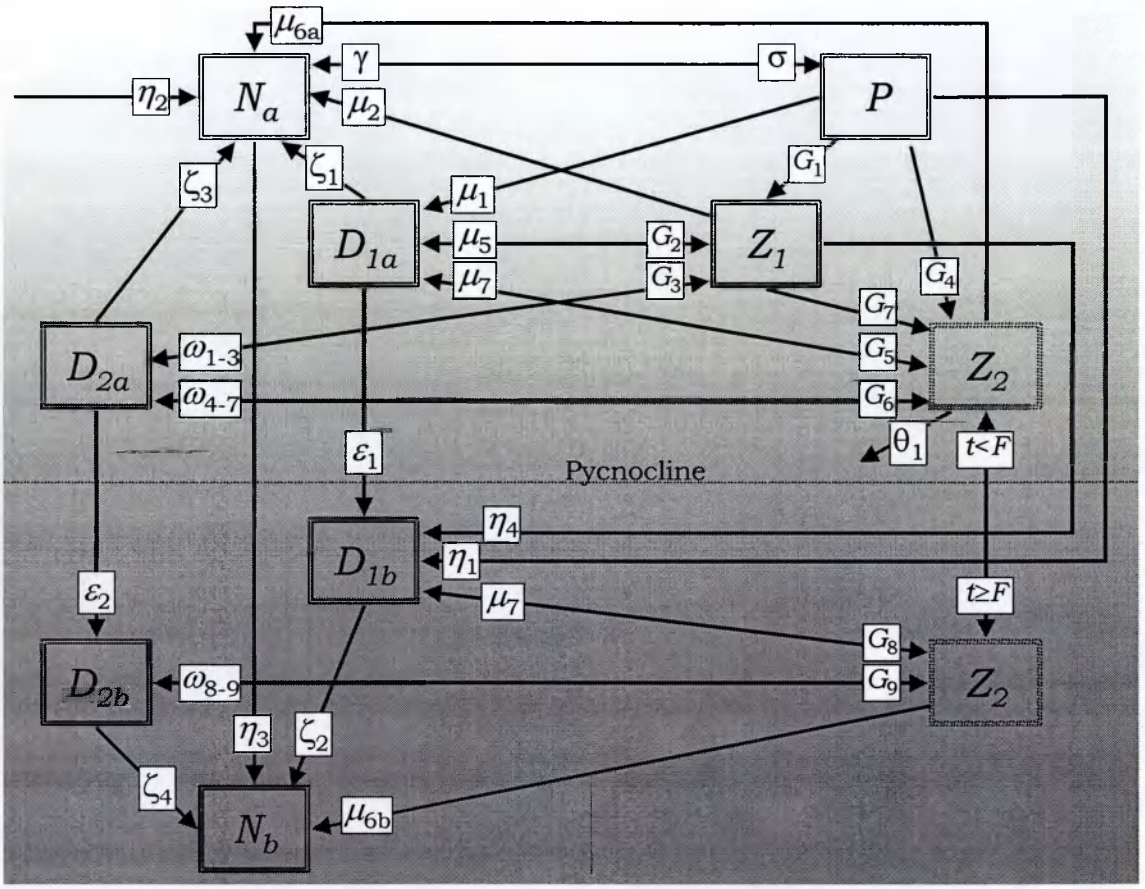

Figure 8.1 Diagrammatic representation of a seasonal model of nitrogen-cycling in the Sargasso Sea (M2), modified from an original mixed-layer model (M1: Fasham *et al.*, 1990) to include the diel vertical migration of interzonal zooplankton. Mixed-layer nitrogen exists in six compartments (pools, or state-variables), and deep-layer nitrogen in four compartments: interzonal zooplankton occupy the mixed layer for only a fraction (F) of each day. See text for a description of the symbols. The 44 parameters associated with the intercompartmental fluxes are listed in Table 8.1.

production. In M1, mixed-layer dissolved nitrogen was differentiated into nitrate, ammonium and labile dissolved organic nitrogen, since the intention here was to describe seasonal changes in new production and the *f*-ratio. Both models are similar in that they envisage a single pool for phytoplankton-nitrogen in the mixed layer (P). In M2, zooplankton-nitrogen is represented by two compartments, namely epizooplankton (Z_1), which spend all of their time in the mixed layer, and interzonal migrants (Z_2), which spend a fraction of each day ($t<F$) in the mixed layer, and the remainder ($t\geq F$) in

the deep layer. Only epizooplankton were considered in M1. Where M1 described a single, all-inclusive term for detrital-nitrogen in the mixed layer, two compartments are distinguished in M2, namely dead phytoplankton and zooplankton (D_1), and zooplankton faecal pellets (D_2). This permits a functional distinction to be made between the detrital food sources available to the zooplankton, providing greater flexibility in the model. In the mixed-layer, the detrital pools (D_{1a}, D_{2a}) may be recycled, either through ingestion by zooplankton, or through bacterial breakdown (remineralisation) into N_a, or they may diffuse and sink through the pycnocline to contribute to the deep-layer detrital pools (D_{1b} and D_{2b}). The deep-layer detritus may be either ingested by interzonal zooplankton (when $t \geq F$), or remineralised into N_b. Although a bacterial-nitrogen pool was described in M1, this is not included in M2 at this stage for the sake of simplicity. In both models, the state-variable units used are mMol N m^{-3}, meaning that intercompartmental flows have the units mMol N m^{-3} d^{-1}. These can be converted to areal units of mMol N m^{-2} d^{-1} where appropriate, by multiplying by the concurrent depth (in metres) of the mixed layer. For consistency with the units used throughout this thesis, these are converted to mg N by multiplying by 14 (the relative molecular mass of nitrogen).

8.2.2 Mixed layer depth equation

The change in mixed layer depth (dM) as a function of time, t (days), is written as:

$$\frac{dM}{dt} = h(t)$$

Equation 8.1

Fasham *et al.* (1990) discussed (after Evans & Parslow, 1985) how the volumetric

concentration of a state-variable in the mixed layer will be affected by changes in M. Specifically, when the swimming speeds of plankton are sufficient for them to be unaffected by diffusive processes, individuals can actively avoid diffusive mixing and detrainment out of the mixed layer. Therefore, when M decreases and increases, while the areal concentration (mMol m^{-2}) of the population in the mixed layer will remain the same, its volumetric concentration (mMol m^{-3}) will increase and decrease, respectively. For non-motile entities, an increase in M will again dilute the volumetric concentration. A decrease in M, however, will actually result in some material being left behind at depth (detrained), such that the areal concentration in the mixed layer will be reduced, while the volumetric concentration will in fact remain unchanged. This was recognised in M1 by employing $h(t)$ in equations dealing with motile forms of nitrogen, and $h^+(t)$ (representing only positive values of $h(t)$, i.e. when M is increasing) when dealing with non-motile forms. The same solution is adopted in M2, where interzonal zooplankton are assumed to be sufficiently motile, and all other forms of nitrogen, including the epizooplankton, are assumed to be non-motile.

8.2.3 Phytoplankton equation

The pool of phytoplankton-nitrogen in the mixed layer (P) increases through the specific uptake of N_a (the average daily phytoplankton specific growth rate, σ), and decreases through the specific exudation of N_a (γ), zooplankton grazing (G_1, G_4 when $t<F$), specific natural mortality (μ_1) and diffusive mixing and detrainment out of the mixed layer (η_1). As in M1, σ is a function of (1) the amount of photosynthetically active radiation (PAR) available to a cell during the daytime, and (2) the nature of the nutrient (N_a) limitation of growth. However, the calculation of σ in M2 has now been modified (after KJF):

$$\sigma = \frac{L\mu N_a}{K_0 + N_a}$$

Equation 8.2

The amount of PAR available to a phytoplankton cell is accounted for by the term $L\mu$, which is analogous to the term J (the "light limited growth rate") in M1, and represents the potential daily growth of phytoplankton (averaged over the mixed layer depth) based on light levels only. As with J, $L\mu$ is a function of the mixed layer depth (M), the photosynthesis-irradiance relationship (the P-I curve), the day-length (τ), the PAR just below the water surface at noon, the light absorption properties of the water column (k), and the phytoplankton maximum growth rate (V_P). M, PAR and the initial slope of the P-I curve (α) are included within the terms k and y, which are described in more detail below (Equations 8.6 and 8.11). The calculation of $L\mu$ is now somewhat different to that of J (after KJF):

$$L\mu = \frac{2\tau V_P}{k} y \left(\frac{0.049y + 0.556}{(1 + 0.189y) - y^{-k}\left(\frac{0.049y^{-k} + 0.556}{1 + 0.189y^{-k}}\right)} \right)$$

Equation 8.3

Nutrient (N_a) limitation is expressed in terms of the half-saturation constant for N_a uptake (K_0). This is considerably simpler than the analogous term, Q, in M1, which also took into account the preferential uptake of ammonium over nitrate. Zooplankton feeding rates (G_i) are discussed below. The calculations of specific exudation (γ),

specific natural mortality (μ_1) and diffusive mixing and detrainment (η_1) are the same as in M1. η_1 is therefore a function of m, $h^+(t)$ and M, where m is a value that quantifies the diffusive mixing across the pycnocline (m d^{-1}), and the other parameters have already been described.

8.2.4 Zooplankton equations

The epizooplankton (Z_1) are assumed to be able to feed on phytoplankton and detritus, and the interzonal migrant zooplankton (Z_2) on phytoplankton, detritus and epizooplankton (after Schnetzer & Steinberg, 2002b). Increases in the zooplankton-nitrogen pools therefore arise from feeding (G_i), while decreases arise from specific excretion (μ_2, μ_{6a}, μ_{6b}), specific mortality (μ_5, μ_7) and defaecation (ω_i). Furthermore, the epizooplankton are deemed to be sufficiently weak swimmers that they are diffusively mixed and detrained out of the mixed layer (η_4) in the same way as the phytoplankton (η_1). Additional losses to the epizooplankton occur through predation by interzonal migrants (when $t<F$). Conversely, the interzonal migrants are deemed to be strong enough swimmers that diffusive processes and changes in M do not act to remove individuals from the mixed layer (when $t<F$). Instead, changes in M act either to dilute or increase the volumetric concentration (θ_i).

The choice of grazing equations to use in M1 was understandably described by Fasham *et al.* (1990) as a "complex issue", given that any given zooplankton component will be both temporally and spatially variable in terms of its food preferences. In order to avoid over-complication in M2 at this developmental stage of the model, the calculation of zooplankton feeding rates (G_i) has been somewhat simplified (after KJF) pending further empirical parameterisation. The feeding rate of, for example, epizooplankton on phytoplankton (G_1), is now calculated as:

$$G_1 = \frac{g_1 Z_1 P}{K_1 + P}$$

Equation 8.4

where g_1 is the specific growth rate, and K_1 is the half-saturation constant for feeding on phytoplankton. In total, M2 includes nine feeding rates: G_1 to G_3 represent the feeding of epizooplankton on P, D_{1a} and D_{2a}, respectively, G_4 to G_7 the feeding of interzonal migrants (when $t<F$) on P, D_{1a}, D_{2a} and Z_1, respectively, and G_8 to G_9 the feeding of interzonal migrants (when $t \geq F$) on D_{1b} and D_{2b}, respectively. A separate growth rate, g_i, is assigned to each zooplankton compartment (i.e. g_1 for Z_1, and g_2 for Z_2), while each of the nine feeding rates (G_1 to G_9) is assigned a separate half-saturation constant (K_i) and assimilation efficiency (β_i). The defaecation rate (ω_i) from a given food source is therefore calculated as:

$$\omega_i = (1 - \beta_i) G_i$$

Equation 8.5

In M1, there were separate parameters (p_i) that defined zooplankton preferences for different food types. These preferences are also expressed in M2, but are now encompassed in the term K_i (the half-saturation constant), which is specific to each grazing relationship. It is also worth pointing out that, in M1, the net increase in Z caused by feeding was described in a single expression, βG, which took into account both total ingestion and defaecation. In M2, this expression is differentiated into the total increase in Z caused by feeding, G_i, and the loss of a proportion of this increase via defaecation, ω_i, allowing greater flexibility when analysing the results of the model. The

calculations of specific excretion (μ_2, μ_{6a}, μ_{6b}), and the effects of changes in M on motile forms of nitrogen (θ_i), are the same as in M1. For the reasons explained above, θ_i is a function of $h(t)$ and M only. Specific mortality (μ_5, μ_7), however, is now calculated as $\mu_i Z_i^2$ (as opposed to $\mu_i Z_i$ in M1) to express the fact that mortality is likely to be a density-dependent process (e.g. Peterson & Black, 1988). Given that changes in M will not affect interzonal migrants when present in the deep layer, the differential equation for Z_2 when $t \geq F$ does not include the term θ_i. Since no differentiation between nitrate, ammonium and DON is made in M2, the relative amounts of ammonium and DON generated by zooplankton excretion cannot be expressed as in M1, nor the effects of higher predators (this was dealt with in M1 by assuming that a fraction of zooplankton mortality was converted to ammonium, and the remainder instantly removed from the mixed layer).

8.2.5 Detritus equations

The pool of dead phytoplankton and zooplankton in the mixed layer (D_{1a}) increases through the specific natural mortality of phytoplankton (μ_1) and zooplankton (μ_5, and μ_7 when $t<F$), and decreases through ingestion by zooplankton (G_2, and G_5 when $t<F$), remineralisation to N_a (ζ_1) and diffusive mixing, detrainment and sinking out of the mixed layer (ε_1). In the deep layer, this pool (D_{1b}) increases through the diffusive mixing, detrainment and sinking of particles from above (ε_1), the diffusive mixing and detrainment of phytoplankton and epizooplankton from above (η_1, η_4), and the specific mortality of interzonal zooplankton (μ_7 when $t \geq F$). Decreases occur through ingestion by interzonal migrants (G_8 when $t \geq F$), and remineralisation to N_b (ζ_2). The pool of zooplankton faecal pellets in the mixed layer (D_{2a}) increases through zooplankton defaecation (ω_1 to ω_3, and ω_4 to ω_7 when $t<F$), and decreases through ingestion by

zooplankton (G_3, and G_6 when $t<F$), remineralisation to N_a (ζ_3), and diffusive mixing, detrainment and sinking out of the mixed layer (ε_2). In the deep layer, this pool (D_{2b}) increases through the diffusive mixing, detrainment and sinking of particles from above (ε_2), and the defaecation of interzonal migrants (ω_8 to ω_9 when $t\geq F$). Decreases occur through ingestion by interzonal migrants (G_9 when $t\geq F$), and remineralisation to N_b (ζ_4).

The specific mortality (μ_1, μ_5 and μ_7), ingestion (G_i) and defaecation (ω_i) terms have been described above. As in M1, the remineralisation of detritus in M2 (ζ_i) is simply calculated as a proportion (μ_4) of its standing stock, and the diffusive mixing, detrainment and sinking of detritus out of the mixed layer (ε_i) as a function of m, $h^+(t)$, V and M, where V is the detrital sinking rate (m d^{-1}) and the other parameters have already been described.

8.2.6 Dissolved nitrogen equations

The pool of dissolved nitrogen in the mixed layer (N_a), increases through the diffusive mixing and entrainment of deep-layer nitrate into the mixed layer (η_2), the remineralisation of mixed-layer detritus (ζ_1, ζ_3), the specific excretion of zooplankton (μ_2, and μ_{6a} when $t<F$), and the specific exudation of material from phytoplankton cells (γ). Decreases occur through diffusive mixing and detrainment out of the mixed layer (η_3), and phytoplankton specific growth (σ). The pool of dissolved nitrogen in the deep layer (N_b) increases through diffusive mixing and detrainment from above (η_3), the specific excretion of interzonal zooplankton (μ_{6b} when $t\geq F$), and the remineralisation of deep-layer detritus (ζ_2, ζ_4). No processes decrease the deep-layer dissolved nitrogen pool, such that this represents the form in which nitrogen is stored (sequestered) for extended time-periods in the deep ocean. The calculations for η_2 and η_3 are the same as in M1. Both are therefore a function of m, $h^+(t)$ and M, with η_2 also a function of the

concentration of nitrate in the deep layer (N_0). The issues surrounding the calculation of η_2, and the use of a constant value for N_0, are discussed in Fasham *et al.* (1990). The calculations of remineralisation (ζ_1, ζ_3), specific excretion (μ_2, μ_{6a}, μ_{6b}) and specific exudation (γ) have already been described.

8.3 Choice of parameters

This model (M2) contains 44 parameters from which the various auxiliaries and rates are calculated. These are listed in Table 8.1, together with their symbols and the values assigned to them for the standard run. There are noticeably more parameters than the 24 used in M1 (see Table 1 in Fasham *et al.*, 1990: note this does not include the standard astronomical formulae for the derivation of light levels) due to slight changes in the model structure, and the definition in M2 of nine separate values for both β_i and K_i. Seven of the parameters used in M1 are now redundant in M2 following the grouping of nitrate, ammonium, and DON into a single term, and the exclusion of a bacterial-nitrogen pool. While the symbols and numbers of the parameters in M2 have been kept as close as possible to those in M1, there are a few variations for the sake of consistency in M2.

8.3.1 Physical parameters

As in M1, ecosystem seasonality in M2 is driven by seasonal changes in M and solar radiation. The values for M used in M2 are from the BATS site (31.7 °N, 64.2 °W) in 2000, and are shown in 8.2a. These were kindly provided courtesy of the BATS programme (P. Lethaby & R. Johnson, pers. comm.: M was calculated using a variable σ_t criterion, after Sprintall & Tomczak, 1992), with the depth being linearly interpolated between the values at each ~monthly sampling time-point. Related to M are the

Parameter	Symbol	Value	
		M2	M1
Initial slope of P-I curve	α	0.025 $(W\ m^{-2})^{-1}\ d^{-1}$	0.025
Zooplankton assimilation efficiency	β_1 to β_9	70 % (in all cases)	75
Cloudiness	C	40 %	4 Oktas
Frac. of each day spent in mixed layer by Z_2	F	40 %	-
Epizooplankton maximum growth rate	g_1	0.6 d^{-1}	1
Interzonal zoop. maximum growth rate	g_2	0.5 d^{-1}	-
Phytoplankton exudation fraction	γ	5 %	5 %
Half-saturation for phyto. nutrient uptake	K_0	0.5 mMol N m^{-3}	0.5
Zooplankton half-saturation for ingestion	K_1 to K_9	1 mMol N m^{-3} (in all cases)	1
Light attenuation by phytoplankton	k_c	0.03 m^2 $(mMol\ N)^{-1}$	0.03
Light attenuation due to water	k_w	0.04 m^{-1}	0.04
Cross-pycnocline mixing rate	m	0.1 m d^{-1}	0.1
Phytoplankton specific mortality rate	μ_1	0.0675 d^{-1}	0.045, 0.09
Epizooplankton specific excretion rate	μ_2	0.15 d^{-1}	0.1
Detritus specific breakdown rate	μ_4	0.05 d^{-1}	0.05
Epizooplankton specific mortality rate	μ_5	0.082 d^{-1}	0.05
Interzonal zoop. specific excretion rate	μ_{6a}, μ_{6b}	0.125, 0.101 d^{-1}	-
Interzonal zoop. specific mortality rate	μ_7	0.02982 d^{-1}	-
Deep-layer nitrate concentration	N_0	2 mMol N m^{-3}	2
Factors for the timing of sunset	a_{set}, b_{set}, c_{set}	0.25, -0.04, 0.009	?
Factors for above-cloud sunlight at noon	a_{sun}, b_{sun}, c_{sun}	1154.5, -253.2, -99.6	?
Detrital sinking rate	V	1 m d^{-1}	1, 10
Phytoplankton maximum growth rate	V_P	1.5 d^{-1}	2.9

Table 8.1 The parameters used in M2, their symbols, and the values assigned to them in the 'standard' run of the model. Also provided is a comparison with the standard-run values assigned in the original model (M1) of Fasham *et al.* (1990).

auxiliary functions $h(t)$ and the specific diffusional mixing and detrainment of non-motile material across the pycnocline ($\eta_i = m + h^+(t)/M$). These terms have been described above, and their seasonal cycles in M2 are shown in Figures 8.2b and c respectively.

As described above, a 'photosynthesis term' (y) is used within the calculations of $L\mu$ (the potential growth of the phytoplankton as a function of available light). y is calculated from the equation:

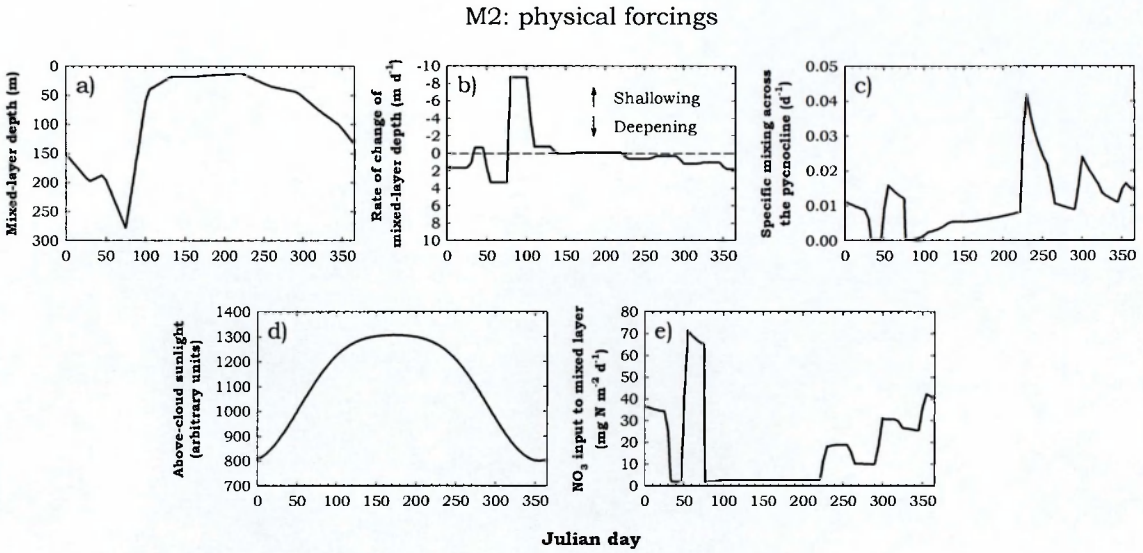

Figure 8.2 The physical forcings in M2: seasonal changes in a) mixed layer depth = M, b) the rate of change of $M = h(t)$, c) the specific diffusion and detrainment of non-motile material across the pycnocline = $m+h^{+}(t)/M$, d) the level of sunlight above the clouds at midday = I, and e) the entrainment of external nitrate (NO_3^-) into the mixed layer = η_2.

$$y = \frac{\mathrm{PAR}\alpha}{\tau V_P}$$

Equation 8.6

Seasonal changes in photosynthetically-active radiation (PAR) are calculated as a function of the average percentage cloud cover in the Bermuda region (C), and the level of sunlight above the clouds (I), according to the equation:

$$\mathrm{PAR} = 0.375I(0.3C)$$

Equation 8.7

Seasonal changes in I (Figure 8.2d) are calculated as a function of latitude-specific standard astronomical formulae for the derivation of above-cloud light levels (a-c_{sun},

M.J. Fasham, pers. comm.):

$$I = a_{sun} + I_s(b_{sun} + I_s c_{sun})$$

Equation 8.8

where I_s, a standard cosine function describing seasonal changes in light for any location, is calculated as:

$$I_s = \cos\left(\frac{\pi}{365}(2\ (t+10))\right)$$

Equation 8.9

Day-length (τ) at a specified location is calculated as a function of latitude-specific standard astronomical formulae for the derivation of sunset (a-c_{set}, M.J. Fasham, pers. comm.), and I_s, according to the equation:

$$\tau = a_{set} + I_s(b_{set} + I_s c_{set})$$

Equation 8.10

The amount of light absorbed by the mixed-layer water column (k) is calculated as a function of the depth of the mixed layer (M), the light attenuation caused by Sargasso Sea water (k_w) and the light attenuation caused by the presence of phytoplankton (k_c, a phytoplankton self-shading parameter):

$$k = M(k_w + Pk_c)$$

Equation 8.11

The values for k_w and k_c used in M1 were also adopted in M2. The reasons for choosing these values are discussed in Fasham *et al.* (1990). Similarly, the calculation of the diffusion of external nitrate into the mixed layer (η_2: Figure 8.2e), and the choice of values for m and N_0, were the same as in M1.

8.3.2 Phytoplankton parameters

A lower phytoplankton maximum growth rate (V_P) of 1.5 d^{-1} was used in M2 (after KJF). The values chosen for α, k_c and γ, and the reasons for these choices, are the same as in M1. Given that nitrate and ammonium were not differentiated in M2, the half-saturation parameter for phytoplankton N_a uptake (K_0) was taken as the mean of the half-saturation parameters for nitrate and ammonium uptake given in M1 (i.e. 0.5 mMol N m^{-3}). The value for specific natural mortality, μ_1, was the mean of the range used in M1 (i.e. 0.0675 d^{-1}).

8.3.3 Zooplankton parameters

Fasham *et al.* (1990) discussed the problems inherent in assigning parameter values to a zooplankton compartment that, in reality, will contain a diverse assemblage of species. The differentiation of two compartments in M2 (i.e. Z_1 and Z_2) goes some way towards addressing this issue. As discussed in section 5.1.5, bacterioplankton and nanozooplankton dominate the heterotrophic carbon-biomass in the mixed layer at BATS, so that much of the mixed-layer nutrients are recycled through the microbial loop. However, given that the objective of M2 is to investigate the active flux, the focus here will be on the mesozooplankton (>0.2 mm), since these assume an importance in the vertical export of material that is disproportionate to their relative biomass (e.g.

Michaels & Silver, 1988). In accordance with previous findings at BATS (Steinberg *et al.*, 2000; Madin *et al.*, 2001), the epizooplankton (Z_1) in M2 are envisaged to consist primarily of individuals in the 0.2 to 2 mm size range, and the interzonal migrants (Z_2) primarily of individuals in the 2 to 5 mm range.

In a recent paper, Roman *et al.* (2002) provided values for a number of mesozooplankton parameters at BATS. They estimated the specific growth rates of a range of size-fractions using the empirical model of Hirst & Lampitt (1998), which assumes that growth is positively related to temperature, and negatively related to body size. Based on this model, the specific growth rate of the epizooplankton (0.2-2 mm) was calculated as 0.11 d^{-1}, and that of the interzonal migrants (2-5 mm) as 0.04 d^{-1}. However, using such low values in M2, in conjunction with the loss rates provided below (mortality, defaecation, and excretion), resulted in both zooplankton components quickly going extinct. Significantly higher values were required in order for the zooplankton to survive: therefore 0.6 d^{-1} was used as the standard value used for g_1, and 0.5 d^{-1} as the value for g_2.

From changes in mesozooplankton biomass over time, and taking into account their estimated growth rates, Roman *et al.* (2002) also calculated a mean mesozooplankton mortality rate of 0.93 mMol C m^{-2} d^{-1} for the top 150 m. From their mean total biomass value in the same depth stratum of 11.38 mMol C m^{-2}, this equates to a mean specific mortality rate of 0.082 d^{-1}. This is therefore the standard mortality value applied here to both μ_5 and μ_7. When calculating defaecation rates, Roman *et al.* (2002) assumed a general assimilation efficiency of 70 % (after Conover, 1978), and this is the standard value applied here to β_i.

The dissolved-nitrogen excretion rates of one of the dominant interzonal zooplankton migrant species at BATS, *P. xiphias*, were given in section 7.2.1 (after Steinberg *et al.*,

2002). It was calculated that excretion in the warmer mixed layer (23 °C) occurred at a rate of 0.52 % body N h^{-1}, and in the cooler deep layer (18 °C) at 0.42 % body N h^{-1}. These values translate into specific excretion rates of 0.125 and 0.101 d^{-1}. In M2, these are the standard values assumed for μ_{6a} and μ_{6b}, respectively. In M1, Fasham *et al.* (1990) mentioned a mean specific excretion rate for a number of Sargasso Sea copepod species of 0.15 d^{-1} (after Verity, 1985). While a lower value than this was adopted in M1, due to problems with the zooplankton going extinct in the winter, this will be the standard value adopted in M2 for the specific excretion rate of the epizooplankton, μ_2.

The chosen values for the half-saturation constants for ingestion (K_i) were initially set at 1 mMol N m^{-3} in all cases, after the value used by Fasham *et al.* (1990) for the epizooplankton. However, as discussed above, the grazing equations used in M2 are simplistic at this stage, and require further empirically based parameterisation in order to describe accurately the spatio-temporal variability in the relationships between ingestion and food concentration.

8.3.4 Detritus parameters

The values chosen for V and μ_4 are the same as in M1.

8.3.5 Numerical methods

As in M1, a fourth-order Runge-Kutta (fixed step) algorithm was used to solve the differential equations in M2. Runge-Kutta methods entail a more sophisticated procedure (than, for example, Euler's algorithm) for estimating the change in a state-variable over a given *dt*, thus providing a higher degree of accuracy. As the name suggests, four flow calculations are made within a given *dt*, and a final function value calculated. A weighted average of these calculations is used as the estimate for the

change in the state-variable. Also, as in M1, a time step of 0.2 d was used, and the data taken from the third year of all simulations (i.e. days 730 to 1095). The reasons for these procedures are discussed in Fasham *et al.* (1990). The model was also 'leak tested' by ensuring that, when the input of external nitrate (N_0) was switched off, the total amount of nitrogen in the system remained constant. This calculation also took into account any changes in the volumetric concentration of motile state-variables in the mixed layer caused by changes in M (i.e. θ_i).

8.4 Model results

8.4.1 Standing stocks

Standard run

The seasonal changes in the standing stocks of mixed- and deep-layer nitrogen from the standard run are shown in Figures 8.3 (mixed layer) and 8.4 (deep layer). For the mixed layer (Figure 8.3), the model predicted high concentrations of dissolved nitrogen and

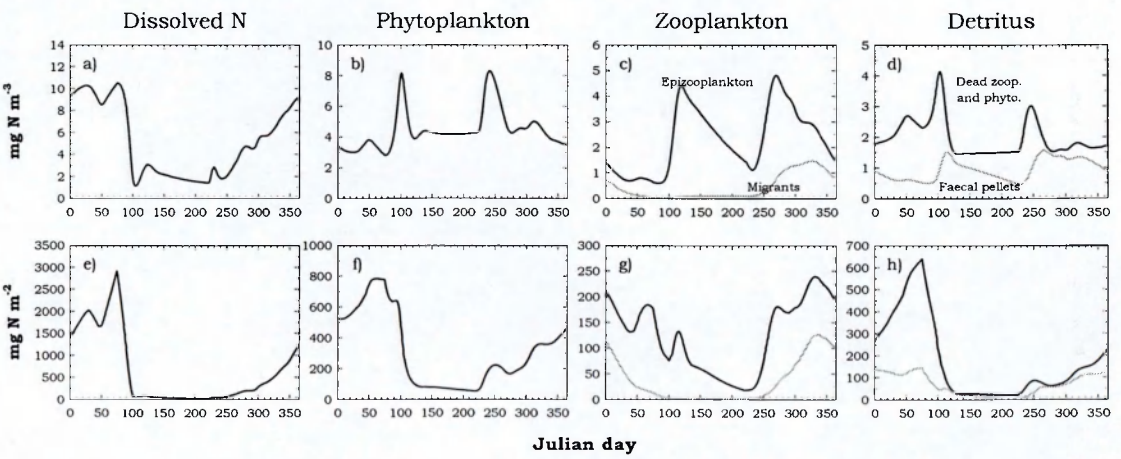

Figure 8.3 Seasonal changes in the standing stocks of nitrogen in the mixed layer predicted from the 'standard' run of the model (using the parameter values given in Table 8.1). Graphs a) to d) show the volumetric concentrations (mg N m^{-3}), while graphs e) to h) show the areal concentrations for the whole mixed layer (mg N m^{-2}).

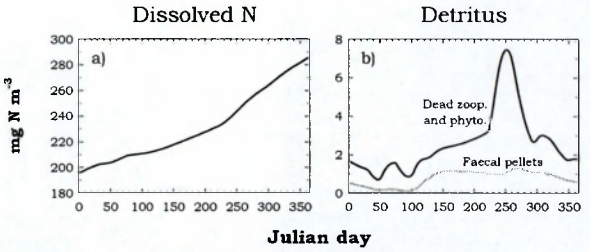

Figure 8.4 Seasonal changes in the standing stocks of nitrogen (mg N m^{-3}) below the mixed layer predicted from the 'standard' run of the model (using the parameter values given in Table 8.1).

low levels of phytoplankton during the winter and early spring (November to March), while the situation was reversed from late spring through to autumn (April to October). A spring phytoplankton bloom was triggered by the winter decrease in zooplankton concentrations (Figure 8.3c and g) and the increasing levels of solar irradiance (Figure 8.2d), in conjunction with high levels of nutrients (nitrate) entrained into the mixed layer (Figure 8.2e) following deep winter mixing (Figures 8.2a-c). As the mixed layer shallowed to ~10 m during the summer (Figure 8.2a), the entrainment of new nitrate was almost completely halted (Figure 8.2e) and a lower level of phytoplankton growth supported almost entirely by regenerated nitrogen (i.e. nitrogen recycled in the mixed layer via zooplankton metabolism and detrital remineralisation). An autumn phytoplankton bloom was triggered by an increase in new nitrate input (Figure 8.2e) caused by the gradual deepening of the mixed layer (Figure 8.2a), at a time when light levels were still high (Figure 8.2d) and zooplankton concentrations low (Figure 8.3c and g). The zooplankton stocks, especially the epizooplankton, responded relatively well to changes in both phytoplankton biomass and foodweb structure (i.e. the relative proportions of phytoplankton, zooplankton and detritus). As one might expect, the levels of detritus (Figure 8.3d) reflected the seasonal changes in phytoplankton and zooplankton biomass. For the deep layer (Figure 8.4), dissolved nitrogen accumulated steadily over time (Figure 8.4a), given that no sinks are modelled for this compartment,

while pulses in the levels of detritus (Figure 8.4b) were linked for the most part to the levels of production in the mixed layer (Figure 8.3b-d). However, there was no pulse of dead phytoplankton and zooplankton following the spring bloom, and this is most likely attributable to the fact that the mixed layer was deeper at this time than in the autumn (Figure 8.2a).

8.4.2 Export fluxes

Standard run

The seasonal changes in the passive and active export of nitrogen out of the mixed layer from the standard run are shown in Figure 8.5. The relative contribution by each pathway to the annual total export is shown in Figure 8.6. These observations are

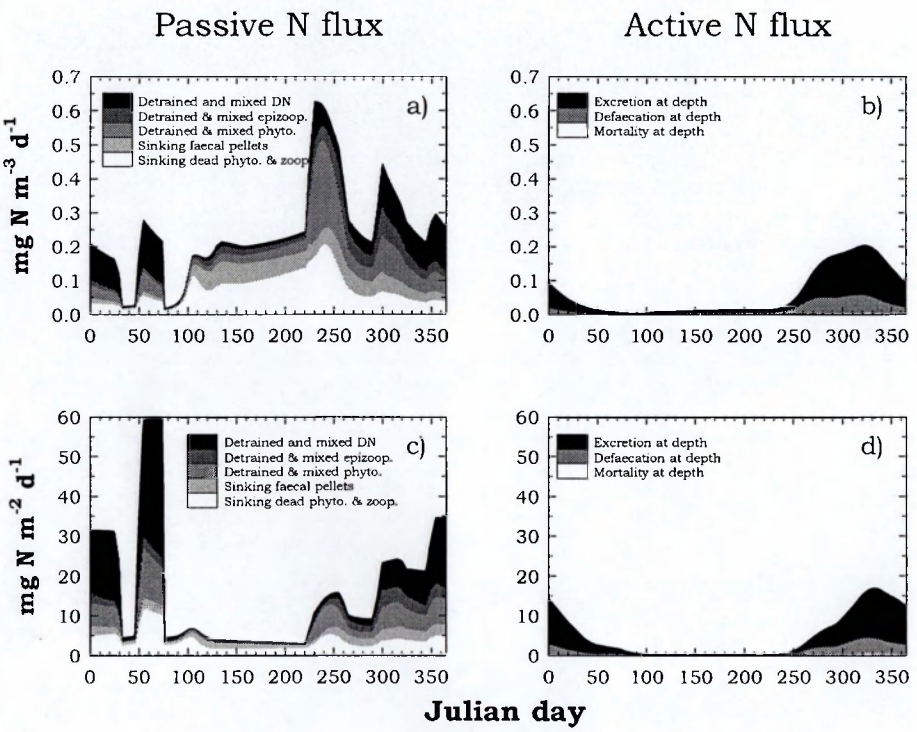

Figure 8.5 Seasonal changes in the passive and active vertical export fluxes of nitrogen out of the mixed layer predicted from the 'standard' run of the model (using the parameter values given in Table 8.1). Graphs a) and b) show the cumulative volumetric fluxes (mg N m^{-3} d^{-1}), while graphs c) and d) show the cumulative areal fluxes across the pycnocline (mg N m^{-2} d^{-1}).

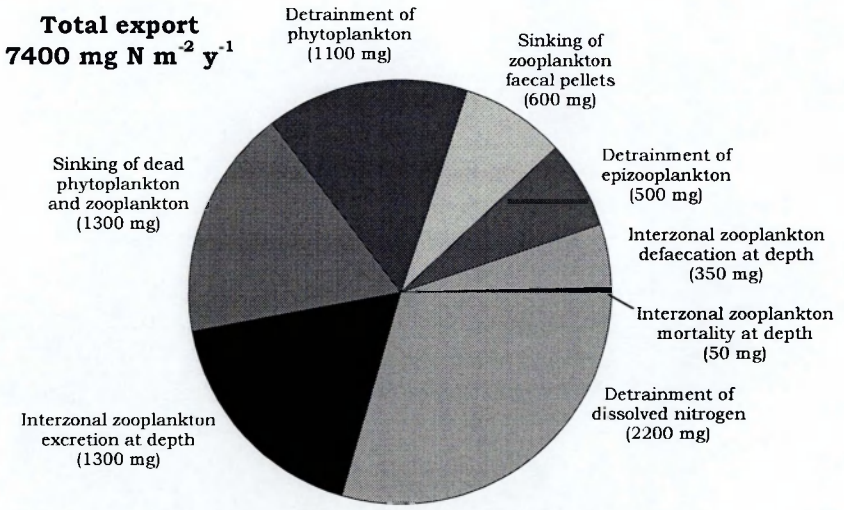

Figure 8.6 The relative contribution of each of the export-flux pathways to the total annual vertical export of nitrogen from the mixed layer (mg N m^{-2} y^{-1}), as predicted from the 'standard' run of the model (using the parameter values given in Table 8.1).

consistent with many of the results presented in the literature regarding the relative importance of the various export-flux pathways.

Regarding the passive flux (Figure 8.5a and c), the export of dissolved nitrogen was of particular significance during periods of deep winter mixing, supporting the field observations of Carlson *et al.* (1994), while the sinking of dead phytoplankton and zooplankton appeared consistently to be the most significant route by which material was exported to depth, as repeatedly suggested in the literature (see section 1.2.4). Interestingly, the sinking export of zooplankton faecal pellets produced in the mixed layer, a pathway which Schnetzer & Steinberg (2002a) suggested to be of importance at certain times of the year, was not significant at any time of the year according to the model. While it is apparent that the complex interactions of the zooplankton must be more comprehensively addressed before we can say too much more on this subject, it is noteworthy that, even during periods when the model predicted high concentrations of zooplankton, the sinking pellet flux was still relatively low. This suggests that a large proportion of faecal material is actually recycled (ingested and/or remineralised) within

the mixed layer. Also of note is the relative importance of the detrainment of phytoplankton out of the mixed layer at certain times of the year, a potential export flux that has received little attention in the literature. Indeed, if the equations presented here (after Fasham *et al.*, 1990) are accurate, then this might well be another important export route worthy of consideration in global budgets.

Regarding the active flux, the often-stated and intuitive link between migrating biomass and export (see section 1.2.4) was well demonstrated here, and the excretion of dissolved nitrogen shown to be of far greater significance than either defaecation or mortality (agreeing with many others, e.g. Hays *et al.*, 1997b). Furthermore, at this early stage, the model provides encouraging evidence that interzonal migrants can significantly enhance oceanic export fluxes via their DVM behaviour, and promises the capability to predict and quantify the importance of the active flux under a range of environmental scenarios.

Sensitivity analysis

In addition to the standard run, 12 further runs were carried out, each with a single parameter value increased from its standard run value (given in Table 8.1). The effect of these parameter increases was monitored in terms of the changes occurring in the various export-flux pathways (Table 8.2). A normalised sensitivity of 2, for example, would mean that for every 1 % increase in the given parameter, there would be a 2 % increase in the flux, and so on (and *vice versa* for negative sensitivity values). It is evident that each of the parameters investigated exerted different effects on each of the export pathways, dependent upon the role of that parameter within the functioning of the model. It is not the intention at this stage to investigate these changes in detail, merely to point out some of the more interesting patterns.

Parameter	Normalised sensitivity								
	ε_1	ε_2	η_1	η_4	η_3	μ_7	$\omega_{8\text{-}9}$	μ_{6b}	Total flux
g_2	0.5	-0.5	0.3	-3.4	-1.1	38.0	9.3	11.4	2.1
N_0	-0.1	0.9	0.2	0.6	1.0	28.9	4.3	5.1	1.6
F	0.4	-0.3	0.2	-1.7	-0.4	19.9	4.0	5.5	1.1
β_i	-0.8	-2.5	-0.5	0.3	0.2	14.7	-0.2	4.9	0.6
μ_7	0	0	0	0	0	0.7	-0.1	-0.1	0
V	0.3	-0.1	0.0	-0.1	-0.1	-0.1	-0.1	-0.1	-0.003
C	0.0	-0.1	0.0	-0.1	0.1	-0.9	-0.5	-0.5	-0.1
k_w	0.2	-0.8	-0.3	-0.7	0.4	-2.0	-1.9	-1.9	-0.4
K_i	1.4	-0.1	0.8	0.3	-0.3	-8.2	-5.3	-5.4	-0.9
μ_{6a}	-0.1	0.1	-0.1	1.3	0.5	-9.1	-6.6	-6.9	-1.4
μ_{6b}	-0.1	0	-0.1	1.5	0.5	-9.7	-7.9	-7.8	-1.6
g_1	-1.6	-0.1	-1.2	2.1	1.2	-9.9	-8.7	-8.7	-2.0

Table 8.2 The normalised parameter sensitivity of the various export-flux pathways for each of 12 parameters investigated (see text for explanation).

The total export flux was noticeably enhanced by increases in the interzonal zooplankton growth rate (g_2), the concentration of nitrate below the mixed layer (N_0), and the fraction of each day spent by interzonal zooplankton in the mixed layer (F). In each case, this increased export was mainly the result of increases in the active flux caused by a greater migrating biomass. This link is easy to understand with regards to g_2 (more growth = greater biomass), and shows that the growth rate is an important parameter to quantify accurately (recall that the value used here was ~10 times higher than suggested in the literature). As for N_0 and F, an increase in each of these will act to increase the migrating biomass by improving productivity (for N_0) and grazing (for F) in the mixed layer. In terms of F, it is perhaps surprising that more time spent in the mixed layer by migrants will actually enhance the amount of material released while at depth. This suggests that the increased migrating biomass caused by the improved grazing and growth allowed in the mixed layer is actually more important in enhancing the active flux than the increased release of material at depth allowed by a greater

fraction of the day spent at depth. Furthermore, this demonstrates the importance of a detailed knowledge of the timing of DVM for any given species when attempting to quantify the active flux.

The total export flux was noticeably reduced by increases in the epizooplankton growth rate (g_1), and the interzonal zooplankton specific excretion rate (μ_{6a} and μ_{6b}). Again, the most sensitive fluxes were those concerned with the active export of nitrogen. In the case of g_1, one might expect that a greater biomass of epizooplankton might help to increase the migrating biomass by providing an increased food source to the interzonal migrants. However, what appears to be happening in the model is that the increased growth rate of the epizooplankton allows them to out-compete the interzonal migrants for phytoplankton and detrital food sources in the mixed layer, therefore actually reducing the success of the migrant population. This is certainly a feasible scenario, and one that the migrants might overcome by switching primarily to carnivory at times when the epizooplankton are prevalent (recall that the model at this stage describes no switching of feeding preferences in line with changes in food quantity and quality). As for μ_{6a} and μ_{6b}, it is perhaps surprising that an increased excretion rate, especially while at depth, does not in fact enhance the active flux. In fact, it appears that the increased loss of nitrogen actually reduces growth, and hence the migrating biomass, and demonstrates the fine balance that exists between ingestion and metabolism, and the success of the population. As discussed above, it is again apparent that the migrating biomass is more important within considerations of the active flux than the specific metabolic rates of individuals.

The total export flux was relatively unaffected by increases in the interzonal zooplankton specific mortality rate (μ_7), the assimilation efficiency of the zooplankton (β_i), the detrital sinking rate (V), the cloud cover (C), the water turbidity (k_w), and the

zooplankton half-saturation values for ingestion (K_i). Certainly the accurate quantification of μ_7, β_i and K_i does not appear to be as important as accurately quantifying g_2 and F. The ability to alter environmental, and potentially climate-related, parameters such as C and k_w promises to afford this model the capability to assess the impact of hypothesised climate changes on the functioning of the marine ecosystem.

8.5 Discussion

It can be seen that M2 has the potential to describe in detail the cycling of nitrogen both within and between the mixed- and deep-layers of the ocean, and that this model promises to be a useful tool in predicting and quantifying the importance of the export fluxes of material (both passive and active) under a range of environmental scenarios. However, it is also apparent that, at this early stage in the model's development, a number of important interactions, particularly those regarding the zooplankton, need to be more comprehensively developed so that, when parameterised with real field data, results are generated that closely match findings from the field. Future developments to M2 might therefore include:

1. Improving the zooplankton grazing equations (G_i) to reflect spatio-temporal changes in the relationships between grazing and food quantity and quality. This is probably the most important aspect of M2 that needs to be developed in the future.
2. Designing a way of having only a fraction of the interzonal zooplankton population (Z_2) performing DVM each day, since numerous zooplankton field studies have shown both daytime residence in surface waters (e.g. Hays *et al.*, 1998) and nighttime residence at depth (e.g. Hays *et al.*, 2002a) in line with individual variability in body condition.

3. Linking the presence of interzonal migrants in the mixed and deep layers to the seasonal changes in daylength, as for the phytoplankton growth equation (σ). The use of a uniform term (i.e. F) throughout the year is somewhat primitive.
4. Incorporating a factor for gut passage time (GPT) into the migrant population so that the true active PON flux can be assessed: the quantification of defaecation at depth provided here (i.e. $\omega_{8\text{-}9}$) is not strictly representative of the active PON flux, and in fact represents the recycling of material ingested at depth.
5. Seeding the phytoplankton and zooplankton populations at various times with advective inputs from other populations, or removing a fraction of these populations via evective losses. This would ideally involve incorporation of M2 into a general circulation model, as successfully carried out for M1.
6. Designing a way of dynamically altering the detrital sinking rate (V) in line with the size-composition of the detrital pools.
7. Adding water temperature as another physical forcing to the model, so that metabolic rates can change dynamically in line with seasonal changes in both mixed- and deep-layer temperatures.
8. Differentiating the dissolved-nitrogen pool (N) into nitrate, ammonium and DON, as in M1, and subsequently adjusting the model equations and links where necessary.
9. Including bacterial-nitrogen, as in M1, and subsequently adjusting the model equations and links where necessary.
10. Differentiating the phytoplankton (P) and zooplankton (Z_1 and Z_2) pools even further to reflect a broader variety of functional groups, and subsequently adjusting the model equations and links where necessary.

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